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OF

URINE.

BY THE SAME AUTHOR.

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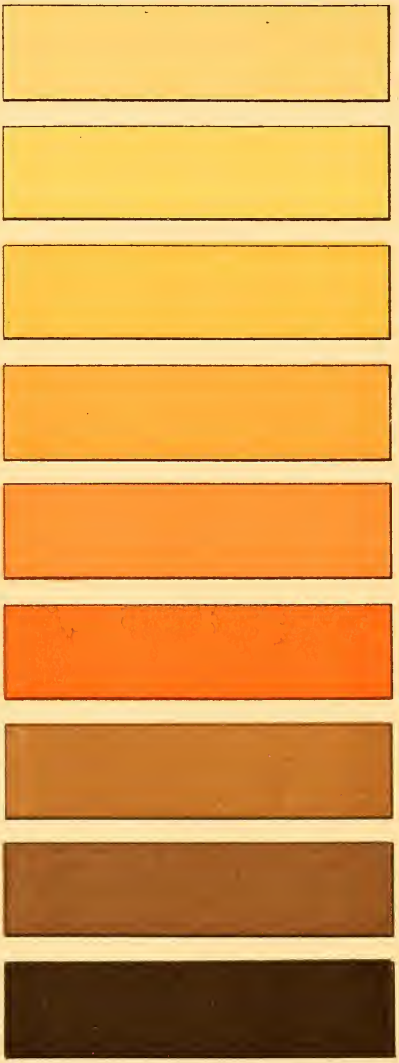

From Nature by Dr. J. Vogel.

VOGEL'S SCALE OF URINE TINTS.

A GUIDE

TO THE

PRACTICAL EXAMINATION

OF

URINE.

FOR THE USE OF PHYSICIANS AND STUDENTS.

BY

JAMES TYSON, M. D.,

Professor of General Pathology and Morbid Anatomy in the University of Pennsylvania; one of the Physicians to the Philadelphia Hospital; Fellow of the College of Physicians of Philadelphia, etc., etc., etc.

SIXTH EDITION.

REVISED AND CORRECTED. WITH A COLORED PLATE AND WOOD ENGRAVINGS.

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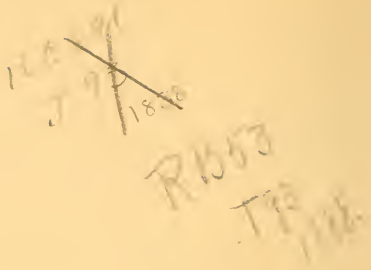

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PREFACE.

It has been the object of the author, in preparing the sixth edition of this manual, to cut down its contents rather than to enlarge them, and considerable matter which appeared in the last edition has been omitted as no longer required. Nevertheless, although great care has been exercised in the admission of new material necessary to keep the book thoroughly modern, the additions have just about occupied the space left by the omissions. At the same time, in view of the enormous amount of literature, good and bad, which had to be sifted, the author congratulates himself on having kept the work to the limits of the fifth edition, which it just about equals in the number of pages.

The most important additions have been the new tests for sugar by phenyl-hydrazin hydrochlorate and by alpha naphthol and thymol, all of which are too recent to have had their true value ascertained.

1506 Spruce St., April 16, 1888. JAMES TYSON.

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INTRODUCTION.

SECRETION OF URINE.

The theory which explains the secretion of urine most consistently with the facts, is one which, while it makes the process partly physical, requires also something of the nature of elaboration in the office of the kidney. Nothing can be more attractive at first thought than the theory of Ludwig, according to which the process is a purely physical one—partly a filtration and partly a diffusion or osmosis. He correctly states that in the capillaries of the Malpighian body the blood-pressure is relatively greater on account of the resistance to the exit of the blood through the efferent vessel. As the result of this, a filtration of the watery constituents of the blood, with some dissolved salts, takes place into the Malpighian capsule. Thus the blood is greatly thickened when it reaches the second capillary network embracing the convoluted tubules into which has descended the thin aqueous filtrate from the Malpighian bodies. Here are the essential elements of a complete osmometer—an animal

membrane formed by a thin wall of the capillary and the delicate basement membrane of the tubule, with a dense fluid, the blood, on one side, and a thin saline solution on the other. An interchange now takes place, as the result of which a current sets in, of the water from the tubules to the blood, and of the products of regressive metamorphosis, urea, etc., and salts, to the tubules, concentrating the fluid in the latter, making it, in a word, urine; while the albuminous constituents of the blood are retained in it because of their well-known indisposition to osmosis.

The objection formerly made to the physical nature of the act of secretion of urine, on the ground that we cannot by this method account for the formation of an *acid* fluid from an *alkaline* one, no longer holds, since Dr. Ralfe, of London, has shown this to be quite possible. Into one limb of a small U-shaped tube, fitted with a membranous diaphragm at the bend, he introduced an alkaline solution of sodium bicarbonate, and into the other limb a solution of neutral sodium phosphate. He then passed a weak electric current through the solutions. In a short time the fluid in the limb connected with the positive pole became acid from the formation of acid sodium phosphate, while the fluid in the limb connected with the negative pole increased in alkalinity. The changes are represented by the following formula:

Sodium bicarbonate.		Neutral sodium phosphate.		Sodium carbonate.		Acid sodium phosphate.
$NaHCO_3$	+	Na_2HPO_4	=	Na_2CO_3	+	NaH_2PO_4.*

One important fact, however, remains unaccounted for by this theory, beautifully simple as it is. This is, that if

* Medical News and Library, October, 1871, from London Lancet July 4, 1871.

the tubules are stripped of their epithelium, as they often are in disease, urea and other products of regressive metamorphosis are no longer so freely removed, but accumulate in the blood, producing the phenomena of the condition known as *uræmia*. We must therefore admit some elaborating action on the part of the epithelium, as originally suggested by Bowman. Doubtless, however, a part of the act is physical—a process of transudation or filtration, and of diffusion or osmosis.

The recent experimental researches of Heidenhain* have settled the question in favor of an active elaborating office on the part of the epithelium of the kidney. Heidenhain injected into the blood of animals, indigo-carmine, a substance which is promptly separated by the kidneys. He removed these organs at suitable intervals after the operation and examined them minutely. In no instance did he find any of the indigo-carmine in the Malpighian capsules, but the cells lining the convoluted tubules and the looped tubes of Henle were filled with it, as was also the lumen of the tubes if the animal was killed sufficiently long after the injection. Similar experiments with urate of sodium showed that it is secreted at the same place and in the same manner.

REAGENTS AND APPARATUS REQUIRED FOR QUALITATIVE AND APPROXIMATE ANALYSIS.†

It is not a matter of very great importance in what form of bottle reagents are kept. They should hold enough—four ounces is a convenient quantity—and be provided with

* Max Schultze's Archiv, vol. x., 1874, p. 1, and Pflüger's Archiv, vol. ix., 1874, p. 1.

† All reagents and apparatus suitable for urinalysis may be obtained of Bullock & Crenshaw, 528 Arch Street, Philadelphia.

ground-glass stoppers for the acids, but the alkalies are better kept in bottles with rubber stoppers. Those required are as follows:

1. Pure colorless nitric acid (HNO_3).
2. Nitroso-nitric acid, the brown fuming nitrous acid of commerce, —nitric acid containing nitrogen tetroxide ($HNO_3 + N_2O_4$ or NO_2).
3. Pure hydrochloric acid (HCl).
4. Pure colorless sulphuric acid (H_2SO_4).
5. Pure acetic acid ($C_2H_4O_2$).
6. Liquor potassæ, U. S. P. The sp. gr. is 1065, and it contains .058 of potassium hydroxide (HKO).
7. Solution of caustic potash, or caustic soda, 1 part to 2 of distilled water, sp. gr. 1330+, to be spoken of in the text as the "stronger solution of potash." It is the ætzkalilauge (or ætznatronlauge, if soda) of the German Pharmacopœia, and contains from .30 to .31 of the hydrate of potassium (or of sodium).
8. Solution of sodium carbonate, 1 part water and 2 parts of the crystallized salt.
9. Solution of barium chloride, 4 parts crystallized barium chloride, 16 of distilled water, and 1 of hydrochloric acid.
10. Liquor ammoniæ, U. S. P.
11. *The magnesian fluid*, containing of magnesium sulphate and pure ammonium chloride, each 1 part, distilled water 8 parts, and pure liquor ammoniæ 1 part.
12. Solution of copper sulphate, say 1 gram to 30 c.c., or 15 grs. to f℥j.
13. Pavy's or Fehling's copper solutions, made as directed under volumetric analysis for sugar.
14. Solution of silver nitrate, 1 part to 8 of distilled water.
15. Solution of neutral lead acetate (sugar of lead), 1 part to 4 of distilled water.
16. Solution of basic lead acetate, 1 part to 4 of distilled water.
17. Distilled water, a liter or a quart.
18. Alcohol, 95 per cent., half a liter or a pint.

Other solutions as required.

Apparatus.

A note and drawing-book.

1 dozen test-tubes, assorted sizes, some narrow. Some test tubes with bases, so that they may stand on a shelf or table, are convenient and desirable; see Fig. 5. Some tubes should be graduated in divisions of a centimeter and fractions thereof. They may be used as fluid measures, and to determine the proportion of a sediment, or of albumin after its precipitation by heat.

Test-tube rack and drainer.

4 conical glasses. (Observe that there is not a convexity at the bottom, and the edge should be ground so that they may be covered with ground glass covers and thus made air-tight.)

2 or 3 smooth wineglasses, with broad bottoms, of the kind sometimes known as "collamore" wineglasses.

Red and blue litmus paper; filtering paper.

Urinometer and urinometer glass.

4 ground-glass covers, assorted sizes.

Spirit-lamp.

3 porcelain capsules.

6 beaker glasses, small and medium sizes.

6 watch-glasses.

3 glass funnels, assorted sizes.

1 long narrow funnel tube 12 inches long and 1 inch wide, for filtering through animal charcoal.

Glass stirring-rods and plain glass pipettes.

1 large receiving-glass to measure twenty-four hours' urine, with capacity of 2000 cubic centimeters or more.

1 graduated measuring-glass holding 500 c.c.

1 wash-bottle with distilled water.

1 retort stand; water-bath.

1 or 2 sheet-iron tripods with wire gauze to cover.

1 100-minim pipette; 1 volume pipette for 5 c.c., another for 10 c.c.

Platinum spoon; tongs.

Blowpipe.

Swabs for cleaning test-tubes, etc.

A microscope with two object-glasses, a $\frac{1}{4}$ or $\frac{1}{5}$ inch, and a 1 inch or $\frac{8}{10}$ inch; stage micrometer; camera lucida for drawing; glass slides, thin covers, *shallow cells;* test-bottles with capillary stoppers.

For volumetric analysis are required in addition:

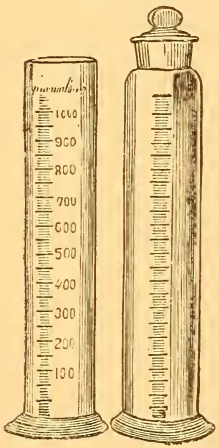
A full set of volume pipettes, 5, 10, 15, 20, 30, 50 c.c.
1 dropping pipette holding 1 c.c., graduated in $\frac{1}{10}$ths and fractions thereof.
2 burettes of 50 c.c. capacity; burette stand.
A half-liter flask.
Volumetric solutions as directed under Volumetric Analysis.

If the solutions are made by the student himself, as they may be, he should be provided with a balance which will turn with a milligram, or with $\frac{1}{50}$ of a grain if the English system is used.

SELECTING A SPECIMEN OF URINE.

In obtaining a specimen of urine for examination, it should, as far as possible, be a part of the whole twenty-four hours' urine, as the specific gravity, reaction, and other properties are well known to vary during the twenty-four hours, and the only accurate method is, therefore, to take a part of the total. When this is not possible, circumstances determine the selection. Thus, when a small quantity of albumin is present in urine, it is often increased after a meal, and sometimes when there is no trace apparent in the morning urine, a little will be detectable after a meal. The same is true of sugar, although a very good plan in the case of saccharine urine is to ask for two specimens, one passed on retiring or about an hour after dinner, and the other passed on rising. In Fig. 1 are represented forms of glass vessels used for measuring large quantities of urine.

Fig. 1.



GENERAL PHYSICAL AND CHEMICAL CHARACTERS OF THE URINE.

Normal urine may be described as a transparent, aqueous fluid, of a pale lemon-yellow hue, acid reaction, specific gravity of about 1020 when passed in the average quantity of 1500 cubic centimetres (50 ounces) in the twenty-four hours, and possessing an odor which can only be indicated as "characteristic" or "urinous." The odor is sometimes spoken of as "aromatic."

Each one of these characters is, however, liable to some variation within the limits of health, as well as in disease, and with these variations we should be thoroughly familiar before interpreting a given specimen.

I. **As to Transparency.**—This, although quite a constant, can scarcely be considered an essential character of normal urine, while, on the other hand, it by no means follows that because a given specimen of urine is transparent, it is therefore normal.

Causes of Diminished Transparency. — Diminished transparency may be due to one of *three* causes.

1. Even urine which is apparently perfectly transparent when passed, commonly exhibits, a few minutes after standing, floating somewhere between the top and bottom, a faint cloud, which is said to be *mucus* derived from the genito-urinary tract. In the urine of females this cloud is apt to be more distinctly visible, in consequence of a larger amount of epithelium from the vagina and adjacent mucous surfaces in this sex. There is nothing abnormal in the presence of such an amount of mucus as is covered by the above description. The effect of alkalies, heat, and strong acids is to leave the appearance unchanged, but acetic acid *may* produce a slight increase of the opacity by coagulating the *mucin*.

2. Normal acid urine may be partially opaque at the moment when passed, by reason of the presence of the *earthy phosphates of calcium and magnesium.* These, shortly after passing, begin to subside, and within half an hour, present an appearance not unlike that of mucus,—that of a flocculent mass, floating somewhere between the top and bottom of the vessel. But still later, generally within an hour, they have approached the bottom, and become a sediment, cloudy, and bulky, leaving a transparent supernatant fluid.

To *test* the nature of such sediment add a few drops of any acid, as nitric, which will cause the prompt disappearance of an earthy phosphate, while the application of heat will increase the deposit, such increase being also rapidly dissipated on adding an acid.

The more or less constant presence of the earthy phosphates above mentioned cannot be considered abnormal. Requiring an acid urine to keep them in solution, a diminution of the degree of acidity may result in their precipitation, which is further increased by an alkaline reaction. Such diminished acidity and substitution of alkalinity always takes place during the digestion, and the deposit is therefore commonly observed at such time.

3. Urine is sometimes rendered turbid by the presence of the so-called *mixed urates* of sodium, potassium, calcium, and magnesium. The most frequent cause of this precipitation in normal urine is a reduction in the temperature of the urine after being passed. Although highly soluble in water at the temperature of the body, the urates are promptly precipitated from a cold urine, such as would prevail in a room without fire in winter.

As in the case of earthy phosphates, such opacity soon diminishes by subsidence of the disseminated urates, which

become a white or pink *deposit*, less bulky than that of phosphates; urates are also apt to be precipitated on the *side* of the vessel.

To *test* the nature of this deposit apply heat, which quickly causes the dissipation of urates, while a sediment of phosphates is increased by it.

1. **Pathologically**, urine may be opaque or semi-opaque from abnormal degrees of the above conditions, or from the presence of *pus*, which also subsides with a rapidity inversely as the quantity of mucus. If the latter is absent, or present in small quantity, the subsidence is rapid; if, on the other hand, it is large, subsidence is slow, often requiring several hours. The turbidity of such urine is *increased* by the application of heat and acids, in consequence of the precipitation of albumin which is always a constituent of *liquor puris*.

2. The presence of fat in a state of minute subdivision, as in the so-called *chylous* urine, produces a degree of turbidity ranging from mere cloudiness to absolute milkiness. In such urine the fatty matter is disposed to rise and form a whitish, creamy layer on top of a less turbid fluid. Such condition, not uncommon in tropical countries, is also sometimes met in temperate climates.

II. **As to Consistence.**—In health, urine is never anything else but aqueous, that is, it drops and flows readily, like water.

Pathologically, it often becomes viscid, glutinous, and separable with difficulty into drops, or not at all. Such state may be due to the presence of an excess of pure mucus, or of a mixture of mucus and pus, and very frequently it is caused by the action upon pus of an alkalinity due to the presence of ammonium carbonate. This will be again alluded to.

In the chylous urine above referred to, the presence of the molecular fat also increases the consistence of the urine.

III. **As to Color.**—While normal urine may be characterized in general terms as *pale-yellow*, *lemon-yellow*, or *amber-hued*, there may be considerable variation in health. Due to the presence, in solution, of the normal coloring matters, the color is deeper or paler according to the proportion of water dissolving them. After copious libations of beer or water, the quantity of urine discharged being large, it is very pale. On the other hand, circumstances which diminish the proportion of water within the limits of health deepen the color. The complemental relation of the skin and kidneys is well known. Under the influence of warmth, therefore, when the skin is acting freely, the quantity of urine is smaller, and it is darker. In winter, the skin being less active, the quantity of urine is larger, and its color less deep. In persons from whom the respiratory exhalation is greater, the urine is likewise less abundant, darker, and *vice versa*.

Pathologically, the color of urine may be altered, 1st, by increase or diminution of the normal coloring matters, or 2d, by the addition of abnormal ones.

1. The former is generally due to a change in the proportion of the coloring matters to the watery constituent. Thus we have almost an absence of color in the copious urines of diabetes, hysteria, and convulsions, while the urine of fevers and febrile states is high colored, chiefly because the quantity of water is diminished, but in the latter instance also because of the addition of an abnormal coloring matter known as *uroerythrin*.

2. (*a*) The addition of abnormal coloring matters is seen in the instances just mentioned—fevers, in urines contain-

ing blood or blood-coloring matters, and bile pigment; and in the *blue* and *brown* urines of which instances have been reported.

(*b*) The urine is also colored after the ingestion of certain vegetable matters eliminated by the kidneys, as santonin, which imparts a yellow color.

IV. The **reaction** of normal *mixed* urine, that is, the urine of the entire twenty-four hours, is always acid. And, generally, specimens of urine passed at any time of day exhibit this reaction, though there is a difference in its degree, while after a meal the urine may become neutral or even alkaline.

The cause of this change in the reaction is still disputed. Roberts believes that it is due to an admixture with the blood of the elements of food, which are largely alkaline, and that the resulting increased alkalinity affects the reaction of the urine secreted. Bence Jones contended that it is the demand made on the blood for the elements of the acid gastric juice, which thus affects the reaction of the urine secreted during digestion. While neither explanation is altogether satisfactory, the former seems more likely to be correct.

The cause of the acid reaction of the urine is usually said to be *acid sodic phosphate*, though it is probably also slightly contributed by other acid constituents, as *uric* and *hippuric* acids, and, under certain circumstances, also by lactic and acetic acids.

There is often observed in urine which has been standing for a short time, especially at a moderate temperature, an *increased* degree of acidity, which sometimes results in a decomposition of urates, and a precipitation, first of acid urates, and later of uric acid crystals. This has been as-

cribed by Scherer to an *acid fermentation*, in which, the mucus acting as the ferment, lactic and acetic acids are formed by the decomposition of the coloring matters of the urine. This has not been altogether satisfactorily proven, while the increased acidity is by no means constant.

It is certain, however, that acid urine which has stood for some time, and more rapidly in hot weather, acquires an ammoniacal odor, and becomes alkaline in its reaction; attending this change of reaction is a semi-opacity with a precipitation of a white amorphous and crystalline sediment, and often also with the formation of an iridescent pellicle on the surface. The cause of these changes has been well determined, and has already been alluded to. Through the action of mucus, and other organic matters, acting in their decomposition as a *ferment*, the urea is converted into ammonium carbonate by the addition of two equivalents of water. Thus:

$$CH_4N_2O + 2H_2O = (NH_4)_2CO_3,$$

which gives the odor of ammonia and the alkaline reaction.

The opacity and deposits are due to the precipitation of the crystalline triple phosphate of ammonium and magnesium, the amorphous phosphate of lime, urate of ammonium, and to living vegetable organisms known as bacteria.

The alkalinity thus resulting from the presence of volatile alkali (ammonia) is easily distinguished from that due to fixed alkali (potash or soda). On drying the test-paper, which has been rendered blue by volatile alkali, the blue color disappears, and the paper resumes its original red or violet tint; if due to fixed alkali the blue remains after desiccation.

V. The **specific gravity**, as stated, may be put down at

1020 for an average amount of 1500 c.c. (50 oz.) in the twenty-four hours. But as this amount is by no means fixed, while the amount of solid matter remains about the same, the specific gravity must vary accordingly. When, from the action of cold or other cause, the skin is not acting, and after copious use of water and diuretics, the specific gravity may descend to 1010, and even lower, within the limits of health. But, when perspiration is copious, or a drain of water from the economy takes place through some other channel, the urine becomes concentrated, and may be 1030 or higher in specific gravity.

Pathologically, the specific gravity of urine is increased or diminished, but to be quite reliable, observations should be made on the entire quantity passed in the twenty-four hours. The specific gravity is increased in *diabetes mellitus*, where it sometimes reaches 1050. A specific gravity of more than 1028, if it attend a copious urine, should excite suspicion of diabetes, and calls for sugar tests. In one instance which came under my observation, a specific gravity of 1010, in a specimen of albuminous urine, was attended by the evident presence of sugar, easily shown by all the tests,* proving that it is not safe to infer from a low specific gravity alone the absence of sugar.

The specific gravity is also increased in the first stage of *acute fevers*, in consequence of the increased amount of solid matters excreted; and in the *first stage* of acute

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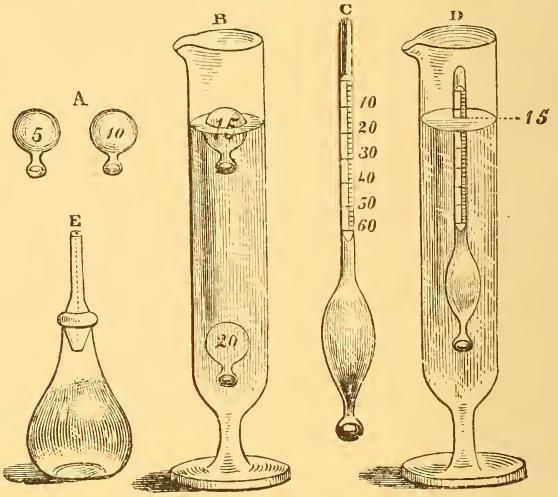
* The first edition of this book noted that I had not met sugar in urine with a lower specific gravity than 1020, but as my experience grew I found it in urines of less weight, until in 1881 I met the instance referred to in the text.

Bright's disease, from the presence of *blood*, the higher specific gravity of the latter and the diminished secretion raising that of the mixed fluid. The specific gravity is diminished in *hysterical* and *spasmodic* hydruria, though here it attends a proportionate increase of water, and is not of much practical significance. In all forms of *Bright's disease*, except the stage of acute nephritis referred to, and

FIG. 2.



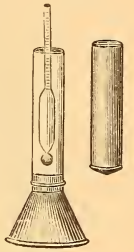
(From Harley.)

in the condition known as cyanotic induration of the kidney, which often attends heart disease, there is a *tendency* to lowering of specific gravity owing to the diminished proportion of urea. Particularly is such reduction of specific gravity significant when it attends diminished secretion of urine. In a general way, the presence of

albumin and sugar being eliminated, variations in the specific gravity point to variations in the amount of urea present; *lower specific gravity of mixed urine* generally *means less urea.*

To determine specific gravity the so-called *urinometer* is almost invariably used, and though less accurate than the picnometer (E, Fig. 2) and balance, is still sufficiently so when carefully constructed. Every urinometer should first be tested with distilled water at 60° F. (15.54° C.), into which it should sink to the mark 0, or 1000. In its graduation the lines indicating the degree should gradually approach each other as the bulb is reached, because allowance must be made for the weight of the stem above water.

Fig. 3.

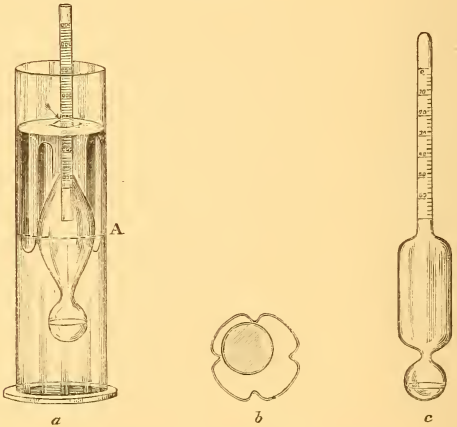


The English-made urinometers, about five inches long (Fig. 2, C), are generally accurate, but the short German instruments (three inches) are very convenient for small quantities of urine. In the little urinometer of Heller (Fig. 3), in which the "sink" consists of leaden shot, the graduation of Baumé is retained, where one degree corresponds with *seven* of the ordinary scale. Thus, 1001 = 1007, 1002 = 1014, and so on. Especial care should be taken in testing these instruments, as a slight variation in them indicates a large one by the ordinary scale. The writer has in his possession an instrument of this kind which recorded the specific gravity of a given specimen of urine 1004, that is, 1028 by the ordinary scale, of which the specific gravity by a long-tried English instrument was found to be 1019. And on testing the former with distilled water it was found to sink, not to 1000, but to 1001 +, proving its inaccuracy. Another urinometer is imported

from Germany even slightly shorter than the original of Heller, in which the ordinary scale is retained on an ivory stem within the tube, and the "sink" contains mercury instead of shot. It is apparently altogether more carefully made, and I have generally found it accurate.

Dr. E. R. Squibb, of Brooklyn, N. Y., has recently suggested a small urinometer standardized for 25° C., or 77° F., a temperature much more usual than 60° F., and at

FIG. 4.



Squibb's Urinometer and jar, shown also in section at *b*.

three points, 1000, 1030, and 1060, with the variations marked, whence corrections are easily made. The glass jar supplied with it is also fluted, and in this way the instrument is kept fron clinging to the side of the glass. This object is further secured by making the air-chamber a double cone, base to base, as seen in *a*, Fig. 4, instead of a cylinder as in *c*. In this way, also, there is obtained

a single point of contact between the urinometer and the jar. Finally, the instrument is provided with a thermometer to secure greater accuracy, but as 4, if water be put at 1000, is about the maximum error which can occur at any temperature at which urine is likely to be tested, it is not really necessary. This is certainly the most accurate urinometer I have seen; and, by reason of the fluting of the glass, a smaller amount of urine is required than in the ordinary perfectly cylindrical jar.

The cylindrical glass vessel usually supplied with the urinometer, or a sufficiently large test-tube, should be about three-fourths filled, the urinometer introduced, and when at rest the specific gravity read off. The cylinder or test-tube should not be too small in relation to the urinometer, lest, in consequence of the capillary attraction between the latter and the walls of the cylinder, the urinometer should not sink as low as it ought. For the same reason the urinometer should not be allowed to impinge against one side of the glass. All these difficulties are provided against in Squibb's urinometer. The scale should be read from above, not below the fluid.

If the quantity of urine be too small sufficiently to fill the cylinder, it may be diluted with distilled water sufficient to fill the cylinder to the required height. From the sp. gr. of this mixture may be calculated that of the urine. Thus: suppose it is necessary to add four times as much water as urine to enable us to use the urinometer, that is, to make five volumes, and the specific gravity of the mixed fluid is 1004, then that of the urine will be $1000 + (4 \times 5) = 1020$. Although the principle of this method is correct, and the results must be, if the data are, the urinometers in use are not usually so nicely graduated that

absolute accuracy in reading is secured: while any error in reading is multiplied by the number of volumes used. Hence it is desirable to use this method as rarely as possible, especially with urines of low specific gravity.

VI. **Quantity.**—The average amount of urine in the twenty-four hours is usually put down at 1500 c.c., or about 50 fluidounces. I am inclined to think this is a little more than the average; perhaps it would be more nearly correct to say between 40 and 50 ounces, or 1200 to 1500 c.c. But enough has already been said to allow the inference that there is also much variation within the limits of health. All that has been said of color and specific gravity in this respect is true of the quantity of urine, though in an inverse ratio. That is in health, *diminished* intensity of color and *diminished* specific gravity correspond with *increased* quantity of urine. It is with regard to quantity that the complemental relation so well known to exist between the skin and kidneys most palpably shows itself, the increased activity of the former causing diminished water separation by the latter, and *vice versa*. In deranged conditions, it is the absence of this relation of color and specific gravity to quantity which gives significance to either.

Pathologically, the quantity of urine is increased in diabetes, and hysterical and convulsive conditions; in the former with increased specific gravity, and in the latter with diminished. In cardiac hypertrophy, in common with all conditions which cause increased blood-pressure, including ingestion of large amounts of water, the peripheral action of cold, etc., there is an increase of water, and a corresponding reduction in specific gravity and color.

In all forms of Bright's disease, except the cirrhotic and lardaceous kidneys, there is a *tendency* to diminished

secretion of urine. Towards the fatal termination, however, it is diminished even in these affections. Any marked diminution of urine in these diseases, particularly if of low specific gravity, which means diminished urea, becomes a grave symptom.

In acute fevers and inflammatory affections, the quantity of urine is very constantly diminished until convalescence sets in, when there is generally observed a marked increase, which, in common with the profuse perspiration often observed at the same time, was long ago characterized by the word "critical."

VII. **Odor.**—Of the odor, little more can be said than that, in health, it is "peculiar" or "characteristic." It is by some spoken of as "aromatic." There is, however, appreciable difference in its intensity, as most have observed in their own cases. Concentrated urines always exhibit what is described in common language as a "strong odor." This is, undoubtedly, due to urea, though the characteristic odor of urine is not ascribed to urea, but rather to the minute quantities of phenylic, taurylic, and damoluric acids found in it.

Urine which has been standing exposed in warm weather acquires an odor which is at once putrescent and ammoniacal, the former from decomposition of mucus and other organic matters, the latter from the ammonium carbonate derived from the urea. The former is predominant when a large amount of organic matter is present, and is often observed in destructive disease of the kidney or its pelvis, and especially of the bladder.

The odor of urine is very promptly influenced by that of substances separated by the kidney from the blood, illustrated by the well-known odor of violets in the urine of persons taking turpentine. The odor of cubebs, copaiba,

and sandalwood oil is promptly communicated to the urine of persons taking them. So, too, the use of certain vegetable foods promptly influences the odor of the urine. Among these asparagus is conspicuous.

Pathologically, except the increased intensity of the characteristic odor of concentrated urines, the *putridity* alluded to, and *sweetish* and *fruity smell* which often attends the presence of sugar in the urine, there seem to be no modifications of the "characteristic" odor of urine except in those extremely rare instances where sulphuretted hydrogen has been found in it.

TO DETERMINE THE AMOUNT OF SOLIDS IN THE TWENTY-FOUR HOURS' URINE.

Knowing the quantity of urine passed in the twenty-four hours, and its specific gravity, an approximation to the quantity of solid matters, and thence that of water, may be readily obtained by multiplying the last two figures of the specific gravity by the coefficient of Trapp—which is 2—or that of Hæser, 2.33. This will give approximately the number of grams of solid matters in the 1000 c.c. (33.8 f. oz.).

Thus, suppose the twenty-four hours' urine to be 1200 c.c. and the specific gravity to be 1022, then, using Hæser's coefficient,

$$22 \times 2.33 = 51.26 \text{ grams in } 1000 \text{ c.c.}$$

But the total quantity of urine in twenty-four hours is 1200 c. c., therefore it will contain more than 1000 c.c. contain. Hence,

$$1000 : 1200 :: 51.26 : x = \frac{51.26 \times 1200}{1000} = 61.51 \text{ grms. (948.09 grs.)}$$

Now, estimating the twenty-four hours' urine at 1500 c.c.,

the normal amount of solid matters is about 70 grams (1080.1 grs.), showing that, in this instance, rather less than the normal quantity was separated. In this manner, valuable information, bearing upon diagnosis and prognosis, may be obtained in a few seconds. The most striking variations are observed in diabetes and Bright's disease. In the former the solids are increased by the addition of sugar, in the latter they are diminished by loss of urea.

While this method of arriving at the solids is not sufficiently accurate for scientific use, it answers for ordinary clinical purposes.

PART I.

THE DIFFERENT CONSTITUENTS OF URINE IN HEALTH AND DISEASE.

In the examination of a specimen of urine, the following are the steps which will be found most convenient in actual practice. Observe—

I. The quantity passed in twenty-four hours.
II. Color and transparency.
III. Odor.
IV. Reaction.
V. Specific gravity.
VI. Presence or absence of sediment, its quantity, and characters.

In all cases, whether the sediment be appreciable or not, a portion of the fluid should be set aside in a conical glass vessel for twelve hours, in order to collect it for *microscopical* examination. The remaining or supernatant fluid, *filtered if necessary*, should then be further examined for certain organic and inorganic constituents.

Organic Constituents.

VII. Presence or absence of albumin and other proteid substances.
VIII. Presence or absence of the different varieties of sugar.
IX. Other saccharine substances.
X. Presence or absence of acetone and diacetic acid.

XI. Coloring matters. { Abnormal. / Normal.
XII. The biliary acids.
XIII. Leucin and tyrosin.
XIV. Fatty matters.
XV. Urea.
XVI. Uric acid.
XVII. Urates.

Inorganic Constituents.

XVIII. Chlorides.
XIX. Phosphates. { *a*. Earthy phosphates. / *b*. Alkaline "
XX. Sulphates.

Examination of Sediment Microscopically and Chemically.

I. Unorganized deposits, including crystals and amorphous deposits.

II. Organized deposits, including anatomical elements, such as tube-casts, epithelium, pus, blood-corpuscles, etc.

III. Other morphological elements, as fungi, granular matter, extraneous substances, etc.

Nos. I, II, III, IV, V, VI, require no further explanation than is involved in the consideration of the "general physical and chemical characters."

Organic Constituents.

VII. Albumin and other Proteids.

Albumin.

The albumin, usually found in urine, is serum-albumin. It and serum-globulin are precipitated from their solutions at a temperature of 73° to 75° C. (163.4° to 167° F.).

This is true of no other of the proteids which occur in urine. Other agencies, however, also throw down serum-albumin, and become equally delicate tests for it, although less reliable, because of their precipitating other substances.

In all instances, where the urine used for testing is not perfectly clear, it should be filtered before applying the tests. This may be done in a few minutes by means of filtering-paper and a funnel.

(a) *The Test by Heat.*

A test-tube is filled to ¼ to ⅓ its depth with *perfectly clear urine*, and heat applied until boiling occurs. If a turbidity result, the slightest degree of which becomes visible in an otherwise clear urine held in a good light, it is due either to *albumin* or *earthy phosphates*. If the latter, it promptly disappears on the addition of a few drops of acetic or nitric acid; if *albumin, it is permanent.* Many years' experience satisfies me that, practically speaking, the results are the same whether the urine is acidified before or after the application of heat, and, as in the vast majority of instances it is already acid, I have here directed the heat to be first applied, one step in the process being thereby saved. Previous addition of acid may also precipitate mucin from normal urine. If further confirmation is desired, to the boiling urine quickly add half as much of the stronger potash solution (7, p. 16), when the albumin is dissolved, and the earthy phosphates again separate in flocculi.

A very good way is to half fill the test-tube with clear urine, and apply the heat only to the upper part, when a resulting diminished transparency can be very easily recognized on comparing the two portions of the tube.

It sometimes happens, even when the precipitate obtained

by boiling is albumin, that the addition of two or three drops of nitric acid will be followed by a partial disappearance of the turbidity, but, if a few more drops be added, the full amount is again thrown down. We should, therefore, continue the addition of the acid until 15 or 20 drops have been used. On the other hand, should the quantity of albumin be very small, too much acid will dissolve it.

If the urine has not been filtered, and is opaque from the presence of amorphous urates, the first effect of the application of heat is to clear up the fluid, and, as the temperature is increased, albumin, if present, is precipitated.

Not only is it true that a small quantity of albumin is dissolved by a large amount of nitric acid, but it should be remembered also that, *if a drop or two of nitric acid be added to a specimen of albuminous urine so as to render it distinctly acid, it may happen on boiling the urine that no precipitate whatever will appear, although much albumin is present.* This is because the serum-albumin has been converted into acid-albumin or syntonin, which is not coagulated by heat. In like manner and for the same reason, albuminous urine boiled in a test-tube in which a drop of nitric acid happens to be present may fail to precipitate its albumin. The same thing may happen when urine is very highly acid from the natural causes of its acidity, although this is rare. Acetic acid, also, may produce out of serum-albumin a soluble acid-albumin not precipitable by heat, if too much acid is used. Hence a drop or two only should be added at first. Whatever be its source, the acid-albumin may be readily precipitated on neutralizing with liquor potassæ. With the least *excess* of alkali the precipitate redissolves, being converted into alkali-albumin.

It is also to be remembered that two or three drops of nitric acid may be added to a specimen of cold albuminous

urine, and although a little cloud of albumin may follow the entrance of each drop into the urine, it will be quickly redissolved, and on the application of heat no precipitate may take place. If nitric acid is used in this way it should be added in considerable excess, yet short of an amount sufficient to dissolve the albumin.

It also occasionally happens, when acetic acid is added to urine naturally acid, and at the same time albuminous, that the albumin is at first only *partially* precipitated on the application of heat, there being a mere opalescence when the quantity of albumin may equal one-half the bulk of urine tested. After waiting a little while, however, the full amount of albumin is thrown down.

Furthermore, serum-albumin is converted by the continued action of an alkali into alkali-albumin, which is also not coagulated by heat. This may occur in highly alkaline urines. But the alkali-albumin is promptly converted into serum-albumin by the addition of a drop or two of dilute acid.

Mehu* calls attention to the fact that urine charged with oxalate of lime becomes slightly turbid when heated, even after all the lime possible has been filtered out, and that the turbidity thus produced is not removed by the addition of a few drops of concentrated acetic acid. I have never encountered this source of error.

(b) *The Nitric Acid Test.*

This is best applied by the contact- or Heller's method. Upon a convenient quantity of pure, colorless nitric acid in a *small* test-tube (one of those with a foot, seen in Fig.

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* L'Urine, Normale et Pathologique, Paris, 1880, p. 326.

5, is most suitable), allow to trickle from a pipette down the side of the inclined glass an equal amount of *clear* urine, which will thus overlie the acid. If albumin is present, there appears at the point of contact, between the urine and nitric acid, *a sharp white band or zone* of varying thickness, according to the quantity of albumin present.

Fig. 5.

Testing for albumin by nitric acid.

The urine may be put into the glass first, if preferred, and the acid may then be allowed to pass down the side and under the urine. The result is the same, but the former is somewhat more easily practiced.

When nitric acid is allowed to underlie *normal* urine,

there appears between the urine and the acid a *brown* ring which grows in intensity on standing, and is due to the action of the acid on the coloring matters. In consequence of this fact, when the urine is highly charged with coloring matters, as it often is in fever cases, the albumin precipitated at the same place is similarly tinted. If there is much indican present in the urine, a rose-red or violet tint may be communicated to the albumin; if much blood-coloring matter, a brownish-red, and if undecomposed biliary coloring matters, a green hue.

Precautions.—1. Much difficulty is often experienced in causing the urine to flow from the pipette with sufficient slowness—that is, it will either not flow at all, or the finger, in the effort to cause it to flow, is raised so much as to permit a sudden fall of the urine into the acid, which interferes with the success of the test. This difficulty is readily overcome by rotating the pipette, covered by the end of the index-finger, between the middle finger and the thumb, whereby the flow may be easily controlled; the process is further facilitated if the upper end of the pipette is slightly *roughened*.

2. A somewhat similar white zone is formed by the action of nitric acid on the mixed urates if present in excess, by which the more insoluble *acid* urates are thrown down. This zone might be mistaken for that of albumin, but the acid urates begin to appear not so much at the border between the urine and acid as higher up; nor does the zone on its upper surface remain so sharply defined, but while under examination is seen to diffuse itself into the urine above. Further, this layer, if caused by urates, is easily dissipated on the application of heat, although some care is necessary in this application lest in ebullition the ring be commingled with the entire mass of fluid and thus lost to view, although not actually dissolved. After some hours have elapsed these amorphous acid urates are completely decomposed by a further action of the nitric acid, and uric acid is then deposited as a characteristic crystalline sediment. Further difficulty arises where, as is occasionally the case in very severe cases of fever, a small quantity of albumin coexists with an excess of acid urates. In these cases the urine is of

high specific gravity, and the line of albumin, lying immediately on the acid, may be obscured by the broader band and cloud of urates. The difficulty from this source is diminished if the urine is diluted with two or three parts of water, while if the method laid down on page 50 is carefully followed out, a mistake is scarcely possible.

It should be added that Thudichum considers that the "cloud" of acid urates here referred to is not urates, but hydrate of uric acid.*

This "diffused haze" brought into view toward the upper part of the column of urine is regarded by Dr. Roberts† as *mucin*. Whether it be acid urates or hydrate of uric acid or mucin, its behavior is very different from that of albumin which appears just above the line of junction of the two fluids.

3. This method of performing the nitric acid test operates equally well with serum-albumin, acid albumin, and alkali-albumin, and therefore obviates the possibility of the source of error referred to on page 37, originally pointed out by Bence Jones—first, that, if albuminous urine be acidified by a small quantity of acid, as a drop or two, no precipitation of albumin takes place; also, that arising from the addition of too large a quantity, as an equal bulk, of acid, when the mixture may in like manner remain perfectly clear. Roberts says he has known the latter fallacy to cause the concealment of albumin in urine for months, in a case of Bright's disease.

4. Occasionally, also, it happens that a urine is so highly concentrated—so highly charged with urea—that the simple addition of nitric acid causes a precipitation of crystals of nitrate of urea. But these are readily distinguished from albumin by their solubility on the application of heat, and by their appearance under the microscope, which exhibits them made up of six-sided rhombic tablets. Such urine is always of high specific gravity, while albuminous urine, except in cases of acute Bright's disease, is apt to be of low specific gravity.

5. If carbonic acid be abundantly present in urine, either free or combined with ammonium, as after the alkaline fermentation, or with

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* Thudichum, J. L. W., Pathology of the Urine, 2d edition, London, 1877, p. 377.

† Roberts on Tests for Albumin in Urine, Medical Chronicle, Oct., 1884, p. 1.

sodium or potassium, during the administration of alkaline carbonates or salts of the vegetable acids, the addition of an acid liberates it with effervescence. Under ordinary circumstances, this does not interfere with the test; but if the quantity of carbonate of ammonium be *very* large, as is the case with some old urines, and the quantity of albumin small, the effervescence is so great as to make the nitric acid test impossible; while the amount of acetic acid required to secure an acidity sufficient to permit the use of the heat test may be so great as to completely hold in solution the small quantity of albumin. Such difficulty is further increased by the fact that these alkaline urines are always more or less cloudy, from the presence of amorphous phosphates and of bacteria, and cannot be cleared up by ordinary filtration. Under these circumstances the following method recommended by Hoffmann and Ultzmann must be pursued. Add to the urine about a fourth part of its volume of liquor potassæ, warm the mixture, and filter. If the filtrate is still not quite clear, add one or two drops of the magnesian fluid (11, p. 16), warm again and filter. The fluid is then always clear and transparent, and albumin, if present, may be revealed by Heller's nitric acid test, or by the cautious addition of acetic acid.

6. Occasionally, after the administration of turpentine or balsam copaibæ, resinous matters are found in the urine. These are precipitated by nitric acid in the shape of a yellowish-white cloud, which is, however, redissolved on the addition of alcohol.

Other Tests for Albumin.

It has long been known that other agents besides heat and nitric acid coagulate albumin. Some of these have lately claimed a large amount of attention, and been found to be extremely delicate tests; some more delicate even than the heat test applied in the most careful manner, and very much more delicate than the nitric acid test. An objection, however, which holds against the most delicate of the reagents is, that they precipitate other substances besides albumin, and although these substances are generally distin-

guishable from albumin by the aid of certain precautions or additional steps, it dare not be said that the reliability of the tests is not weakened thereby.

As the result of a careful study of these tests, based upon experiment and clinical application, I have come to the conclusion that, for the present at least, it is safer to rely upon them in conjunction with the heat and acid tests, with a view to confirming or extending results attained by the latter. Such use is, however, of the greatest importance; and I shall, therefore, now consider the most delicate of them, including the method of their application, and the precautions to be observed in rendering them reliable. Those I deem most worthy of consideration are in the order in which I have found them most delicate: Picric acid, sodium tungstate with citric acid, potassio-mercuric iodide,* ferrocyanide of potassium, and Dr. Roberts's acid brine solution.

Picric Acid.—This has its ablest and most enthusiastic exponent in Dr. George Johnson, of London, who says, "there is no known substance occurring in either normal or abnormal urine, except albumen, which gives a precipitate with picric acid insoluble by the subsequent application of heat."†

An ounce of water at 60° F. retains in solution 5.3 grains of the dry acid. A saturated solution may be made by dis-

* The first three named of these agents are so nearly equal in delicacy that one arranges them in any order at some risk, and I must confess to not invariably having found them delicate in the order named. The experiments of Dr. George Oliver, of London, show that any one of these used in solution by the contact method, will precipitate 1 part of albumin in 20,000 of urine.

† Johnson, Albumen and Sugar Testing, London, 1884, p. 11.

solving 6 or 7 grains of the powder in an ounce of boiling distilled or rain water. A portion of the acid will crystallize out on cooling, leaving a transparent yellow supernatant liquid. Such a solution has a specific gravity of 1005.

Dr. Johnson's mode of applying the test for the detection of a very minute trace of albumin is as follows: Into a test-tube six inches long pour a four-inch column of urine; then, holding the tube in a slanting position, pour gently an inch of the picric acid solution on the surface of the urine, where, in consequence of its low specific gravity, it mixes only with the upper layer of the urine. As far as the yellow color of the picric acid solution extends, the coagulated albumin renders the liquid turbid, contrasting with the transparent urine below. For the action of the test, *there must be an actual mixture*, and not a mere surface contact. When, in consequence of the scantiness of the albumin, the turbidity is very slight, the application of heat to the upper part of the turbid column increases it. Then, if the tube be placed in a stand, the coagulated albumin will gradually subside, and, in the course of an hour or so, forms a delicate, horizontal film at the junction of the colored and unstained strata of urine.

No previous acidulation of the urine is usually required, as the picric acid accomplishes this, if needed. If, however, a specimen be highly alkaline and ammoniacal, Dr. Johnson says the safest method is by acetic or citric acid; then filter and add picric acid to the filtrate. The precipitated mucin will remain on the filter.

Precautions.—1. The urine to be tested should be perfectly clear, and if not clear when obtained should be rendered so by filtration, or the processes described on p. 42.

2. Urates, peptones, vegetable alkaloids, as quinine, morphia, etc., are all precipitated by picric acid solution at the point of contact, but are promptly redissolved by a degree of heat much lower than that of the boiling-point. Quinine promptly appears in the urine after the administration of ten grains of this drug.

Dr. Oliver correctly claims that the addition of citric acid in the proportion of two drachms to an ounce of the picric solution makes a reagent which gives a more distinct reaction than the plain picric acid solution; but Dr. Johnson says that this is because the citric acid precipitates also the *mucin* which exists in all urines.

Dr. Johnson also insists that mucin is not precipitated from urine by picric acid, *and that albumin is the only substance found in the urine which gives with picric acid an opalescence or precipitate insoluble in heat.* He is not sustained in this view by others, notably by Roberts* and Oliver.†

Sodium Tungstate with Citric Acid.—This solution is made by mixing equal parts of a saturated solution of sodium tungstate (1 to 4) and a saturated solution of citric acid (10 in 6). It has a specific gravity of 1214, and is best used by the overlaying method. It is a test of extreme delicacy, precipitating 1 part of albumin in 20,000 of urine, and has the advantage over picric acid of not precipitating quinine from its solution, but, like the picric acid, precipitates acid urates, peptones, and mucin, which are also promptly dissipated by heat.

The Potassio-Mercuric Iodide.—This test was suggested by M. Charles Tanret, of Paris, and is regarded by Dr. Oliver as the most sensitive test known, discovering, like

* Loc. citat., p. 3.

† On Bedside Urine Testing, 3d Ed., London, 1885, p. 111, note.

the picric acid and sodium tungstate, 1 part of albumin in 20,000 of urine. In my own experiments, however, I have several times failed with the mercuric iodide when I succeeded both with picric acid and sodium tungstate. I am inclined to believe that the age of the preparation and the mode of compounding it have something to do with the results.

The solution is prepared by M. Tanret as follows: Bichloride of mercury, 1.35 grams; iodide of potassium, 3.32 grams; acetic acid, 20 cubic centimeters; distilled water, enough to make 1000 cubic centimeters. The resulting reagent is the double iodide of mercury and potassium, the chloride of potassium being without effect. It is also a heavy fluid, having a specific gravity of 1040 +, and is used by the contact method. The urine requires no previous acidulation.

It coagulates the same substances as picric acid, which are likewise dissipated by heat, or the addition of alcohol. On cooling they reappear. With regard to mucin, however, Dr. Oliver says it is not dissipated by heat if a large excess of the reagent be employed, the mercuric salt apparently preventing solution.

Dr. W. G. Eggleston, of Chicago, suggests, with a view to avoiding the confusion of the mucin reaction, that the potassio-mercuric solution be made without acetic acid, and that the urine be acidulated previous to the testing. If a precipitate results, and we have reason to think that it is mucin, half the acidulated urine may be placed in another tube and the mercuric iodide solution or test-paper used. If this causes a further precipitate, as shown by comparison, we may be reasonably sure that albumin is present.

Dr. Eggleston also thinks that, when the solution is made up with acetic acid or any acid, some chemical action must take place sooner or later, as iodide of potassium and bichloride of mercury are comparatively unstable compounds in the presence of an acid.

Ferrocyanide of Potassium.—This test, used in saturated solution, while less delicate than any of the three previously considered, detecting, according to Dr. Oliver, but 1 part of albumin in 10,000 of urine, has the advantage over them of *not precipitating mucin, peptones, or the alkaloids*, but it may throw down acid urates.* It is fully as delicate as nitric acid, but less so than heat. It requires for its operation that the urine shall be acid.

Dr. Roberts's Acidulated Brine Solution.—This solution, which consists of a pint of a saturated solution of common salt to which is added an ounce of hydrochloric acid, and the whole filtered, is about equal in delicacy to nitric acid, but much less so than heat.

It has a high specific gravity, and is used by the contact method. It has the great advantage over nitric acid in that it is less caustic and corrosive, and therefore much pleasanter to work with, while equally delicate.

Roberts's Nitric Magnesian Test.—Dr. Roberts has suggested† another modification of the nitric acid test, consisting of 1 volume of strong nitric acid and 5 of a saturated solution of sulphate of magnesium. This, he says, is more prompt and sensitive than pure nitric acid, and its reaction in regard to albumin, mucin and peptone is similar. It forms a watery, clear solution, which does not fume, nor stain, nor burn the fingers, acts less strongly than the pure acid on the coloring matter of the urine, and may be carried in a corked bottle with less risk of accident. It has received the highest praise from Dr. Henry B. Millard, who says that, as regards delicacy, accuracy and facility of employment, it is the most satisfactory test he has used, detecting less than 1 part in 1,000,000

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* Dr. Geo. Johnson claims that it, in common with all tests which require the combination or addition of acetic or citric acid, does give with mucin of normal urine an opalescence or haziness not distinguishable from that caused by a minute trace of albumin.

† Loc. citat., p. 1.

of water, but he regards as equally sensitive his own test of phenic and acetic acids and Tanret's mercuric iodide. The nitric magnesian fluid has a specific gravity of 1240, and is used by the contact method. It cannot be used with urine clarified by liquor potassæ, on account of decompositions induced. My own experience with it is limited.

Millard's Phenic Acetic Acid Test.—This consists of Acid. phenic, (glacial) (95%) ʒij; Ac. Acet. Pur. ʒvii. Mix, and add liquor potassæ, ℥ij, ʒvi.—Millard claims the test is equally delicate with Tanret's, and the picric acid test, and like them, precipitates mucin, peptone and the alkaloids, and requires the same precautions.

Albumin Test-Papers.—All of these reagents, except the acid brine solution, may be used in the shape of the test-papers suggested by Dr. Oliver, which are more especially useful for bedside testing, although Dr. Oliver claims for them some advantages over the solutions even when used in the laboratory.*

Most recently,† Dr. Oliver has rejected all of the albumin papers except the "mercuric" and "ferrocyanic," and recommends their use as follows:

To Use the Papers.—A mercuric or ferrocyanic, and a citric acid paper are dropped into the test-tube, and water added to 60 minims. After gentle agitation for half a minute or so, the test-papers are removed, and the transparent solution is ready for testing.

The pipette containing the suspected urine is held in a vertical position over the tube, and the urine is delivered in drops. If four drops of urine added to the mercuric

* The different forms of test-papers suggested by Dr. Oliver, originally made for him by Wilson & Son, of Harrowgate, London, are now furnished by Parke, Davis & Co., of Detroit, Mich., as are also, in a single case with the test-papers, the suitably graduated test-tubes.

† Bedside Urine-Testing. London, 1885.

solution, and six to the ferrocyanic,* do not produce a *trace* of milkiness when the contents of the tube are viewed against a dark background, it may safely be inferred that if albumin is present it is in so small a quantity that nitric acid, applied after the contact method for one minute, will not discover it. If a slight milkiness is apparent, it will represent a trace of albumin detectable by nitric acid.

If there is no response, the dropping should be continued, and, if instead of four drops of the mercuric and six of the ferrocyanic solution there be required 10 of the former and 15 of the latter to produce an appreciable opacity, it will indicate a quantity which can readily be shown by heat and acidulation. If 20 of the former and 30 of the latter are required, it indicates a trace of albumin which can be shown only by *careful* acidulation and *subsequent* boiling.

In the case of the mercuric test, if a reaction occurs, the solution should be boiled so as to prove the presence or absence of one of the diffusible proteids, peptone, or hemi-albumose. If the opacity is unaffected by heat, or is intensified by it, it is caused by albumin, but if it is diminished or entirely removed, presumptive evidence is afforded of the presence of a peptoid body, either alone or in conjunction with albumin. The mercuric test, supplemented by heat, may be said, therefore, to provide a fuller knowledge of the proteids which may appear in the urine than the ferrocyanic, which precipitates albumin only.

* The ferrocyanic solution should be allowed a minute in which to develop the reaction from a trace of albumin.

Remarks on Testing for Small Quantities of Albumin. The Author's Method.

To determine the presence of albumin in urine when it is abundantly present is usually a very simple matter. The application of heat will throw down albumin even from an alkaline solution, if the latter is highly charged with it, while the addition of a few drops of acid removes all possibility of error. But it is well known that small quantities of albumin, the significance of which in diagnosis and prognosis is sometimes greater than that of large amounts, often escape detection; while large quantities are sometimes obscured in consequence of peculiarities of combination between albumin and acids, and albumin and alkalies, resulting in the formation of the so-called acid- and alkali-albumins. It is with a view to pointing out the way to avoid such errors that the following paragraphs are written.

Under all ordinary circumstances by far the most striking test for small quantities of albumin is that form of the nitric acid test described as the contact method (p. 38), and in the majority of cases, this test, carefully carried out, even in the hands of the inexperienced, will exhibit the presence of albumin when it would have been overlooked in the ordinary mode of application of heat and nitric acid. But it is *not so delicate a test as the latter* applied in the manner to be described.*

Many who have tested urine for albumin by the ordinary heat and acid test will have observed that after boiling the

* See a paper by the author, "Notes on Albuminuria," in the Proceedings of the Medical Society of the State of Pennsylvania for 1881, p. 644.

clear urine and adding a few drops of nitric acid, the resulting fluid will be apparently clear; but upon setting aside the urine thus treated, say for twelve hours, or until the next morning, there will sometimes be found a small deposit. Supposing the urine before testing to have been *carefully filtered*, this deposit is either, 1st, acid urates; 2d, uric acid; 3d, nitrate of urea; 4th, albumin. The *first* arise from a partial decomposition of the neutral urates by the nitric acid added; the *second* by a further action of the acid upon the acid urates, and a resulting complete separation of the uric acid from the sodium, potassium, etc., with which it was combined; the *third* is found only when the urine happens to be highly concentrated and contains an unusual proportion of urea. The *second* and *third* have well-known forms of crystallization by which they can be easily recognized under the microscope, but the acid urates and albumin are both amorphous and cannot therefore be thus distinguished. *All*, however, *except albumin*, disappear, on the reapplication of heat. In all instances, therefore, urine which has been tried by heat and nitric acid, in which, after cooling and standing from six to twelve hours, a sediment is present, should be boiled again; and if the sediment is not dissolved after such ebullition, it is albumin.

My own method, therefore, of examining a specimen of urine for albumin is invariably as follows:—

I. Unless *perfectly* clear, it is first filtered, and if not rendered clear by filtration, it is clarified by strong alkalies, or the magnesian fluid, according to the directions on page 42. A portion of the filtered fluid is then taken and boiled, being carefully watched in a *good light* for detec-

tion of the least diminution of transparency. A drop or two of nitric acid is then added, and if a turbidity which has ensued upon the action of the heat disappears, it is caused by phosphates of lime and magnesium, and not albumin. The addition of the nitric acid should be *cautiously* continued until a decided excess is added—15 to 30 drops—but not more, lest a small amount of albumin present be redissolved by the excess of acid. If any degree of turbidity remains it is caused by albumin, and the test may end here—although it is well to put the tube aside, in order that the albumin may subside and be approximately estimated. If, however, there is the least doubt about the presence of albumin, the tube must be set away, carefully protected from dust, for six to twelve hours, in order that any appreciable sediment may subside, and be subsequently again tried with heat.

II. A test-tube is now filled to the depth of half an inch with Roberts's acid brine solution or colorless nitric acid. About as much urine is then allowed to fall gently upon it in the manner described on page 39, and the point of junction of the two fluids carefully examined for the white line. This is best observed by holding the tube against a dark ground, produced by a book, pamphlet, or coat sleeve, so that the light may fall obliquely upon the line of junction of the two fluids, while at the same time it is seen against the dark ground.

When this double test is carefully applied as above described, it is scarcely possible to err as to the presence of albumin. Where it is abundantly present, it is, of course, unnecessary to use either the modified heat and acid test or the contact method, although the latter is always useful

in that it affords one means of approximately estimating the amount of albumin.*

Quantitative Estimation of Albumin.

Gravimetric Method.—It is a matter of extreme importance in the course of Bright's disease that we should be able to compare the quantity of albumin contained in the urine from day to day. The only accurate method is by precipitation by acetic acid and boiling, separation by fil-

* The following observation by Dr. C. E. Brown-Séquard, in the first number of his *Archives of Scientific and Practical Medicine* (1873), illustrates some of the difficulties occasionally encountered in this ordinarily very simple process of testing for albumin: "If we first test, by heat, urine containing albumin (after having ascertained that it is naturally acid), we may not find the least precipitate; and if we add nitric acid to it after it has boiled and become somewhat cold, we may yet not find precipitation of albumin. *But if we boil a second time* that now acidified urine, *the solidification of albumin quickly takes place, and a precipitate soon appears.*"

He further says: "In three cases in which the microscope showed tubular casts in the urine, the albumin contained by this fluid was so modified by the heat that if the urine (which was naturally acid) was boiled *first*, the addition of nitric acid in small or in large quantity at a low temperature or at the degree of boiling produced no solidification of that proteid substance. But when I added either a small or a large quantity of nitric acid to the fresh *unboiled* urine and then boiled it, the ordinary coagulation took place, and after some time of rest, the ordinary precipitate appeared. It is evident, therefore, that there is sometimes in the urine a kind of albumin which loses its coagulability by boiling."

The lesson from these facts is that it would seem necessary to apply the heat and acid tests *both ways*, that is, the acid should first be added to the urine and the mixture then boiled, as well as that the urine should be first boiled and the acid then added. I believe, however, if the method above described is carefully carried out, albumin cannot be overlooked.

tration, drying, and weighing by delicately accurate balances, the weight of the filter having been previously determined. This, however, involves too much time for the busy practitioner, and we must fall back on one of the approximative methods.

Approximate Estimation with Boiling. — The easiest of these is to boil a given quantity of urine in a test-tube, add a few drops of nitric acid, and set aside for at least twelve hours—shaking the urine once or twice in this period in order to secure a uniform subdivision and precipitation of the particles of albumin. The proportion of bulk occupied—one-fourth, one-eighth, a trace, etc.—is used to indicate the quantity of albumin. Greater accuracy is obtained by previously filtering the urine of urates, epithelium, or extraneous matter, which might unduly increase the bulk of deposit on standing. Nothing more than a refinement of this is the

Estimation with Esbach's Albuminometer. — This albuminometer consists of a graduated glass tube like that shown in Figure 6. To use it, fill with urine to U, and to R with the test solution, consisting of picric acid, 10 grams; citric acid, 20 grams, and water sufficient to make a liter. Close the tube by a rubber stopper, and mix the contents cautiously, allow the mixture to remain undisturbed for 24 hours, and then read off the quantity of precipitated albumin. Each of the main lines to which the precipitate reaches, indicates one gram of albumin in one liter of urine. The tube may be obtained of Eimer & Amend, New York City.

FIG. 6.

Approximate Estimation with Nitric Acid.—The following method of approximate quantitative estimation by means of Heller's nitric acid method is given by Hoffmann and Ultzmann.

According to them, if the white zone of albumin has the depth of from 2 to 3 mm. ($\frac{1}{12}$ to $\frac{1}{8}$ inch), is delicate and faintly white in color, has no granular appearance, and appears clearly defined only when placed against a dark background, the quantity *is less than* $\frac{1}{2}$ *per cent.*, usually $\frac{1}{10}$ per cent. If the zone is 4 to 6 mm. ($\frac{1}{6}$ to $\frac{1}{4}$ inch) in depth, granular, white, opaque, and perceptible without a dark background, the quantity is considerable, $\frac{1}{4}$ to $\frac{1}{2}$ per cent. If, however, the zone of albumin appears granular and flocculent, and sinks in more or less lumpy masses to the bottom, and when by stirring the albumin by means of a glass rod the mixture assumes the consistence and appearance of sour cream, then the quantity is very large, 1 *to* 2 *per cent.*

Oliver's Approximate Method.—A better quantitative method is that recently suggested by Dr. Oliver. It consists in precipitating all the albumin from a given quantity of urine by a mercuric test-paper, and comparing the resulting diminished transparency with that produced by coagulating the albumin from a standard solution containing $\frac{1}{10}$ of 1 per cent. of albumin. This degree of opacity is imitated by a standard solution made with alumina precipitated by ammonia, which therefore represents $\frac{1}{10}$ per cent. albumin. It is placed in hermetically sealed tubes of the required size. The test is applied as follows: Into a flattened test-tube, graduated to 200 minims, in 10 minim divisions, are poured 20 minims of urine, and a potassio-mercuric iodide test-paper is dropped into the tube. It is then well shaken, and the resulting turbidity is tested by placing behind the tube a card on which lines of various degrees of thickness are printed. If the opacity is such as to obscure the lines completely, water is to be freely added, say to 60 minims in all, the tube again shaken, and the test made with the card. If the opacity is still greater than that of the standard solution, as determined by placing the ruled card behind each, water is to be cautiously added, 10 minims at a time, until the opacity of the mixture corresponds exactly with that of the standard solution. A comparison between the known value of the standard solution and the number of times the urine has been diluted, furnishes the proportions of albumin. Thus the value of the standard opacity being $\frac{1}{10}$ per cent., five dilutions would be .5 per cent., six, .6 per cent., and so on.

When the lines on the card can be read at once, without any dilution, the quantity of albumin is below $\frac{1}{10}$ per cent.

The Proportion of Albumin Found in Urine.—There is much carelessness of expression among physicians in speaking of the quantity of albumin found in a given specimen of urine. Thus we often read that a specimen contains 25 per cent., or even 50 per cent., of albumin. The proportion in bulk is of course intended, but no indication given that this is what is meant. In point of fact 3 or 4 per cent. is probably the maximum amount of albumin which urine can contain, since blood-serum only contains about 5 per cent., and 2 per cent. albuminuria is a very large one. A half per cent. is much more common, and many albuminuric urines contain much less than a half per cent. of the proteid.

It is not unusual, also, to over-estimate the amount of albumin passed in the 24 hours, and thence to exaggerate the drain upon the system. Suppose, for example, the percentage of albumin by weight is .5 of 1 per cent., and the quantity of urine is 50 ounces in the 24 hours, then, there being 455.7 grains in an ounce, $455.7 \times .005 \times 50 = 113.9$ grains, the amount of daily discharge, or about one-quarter of an ounce. Supposing there be 2 per cent., which is a very large amount of albumin, and 40 ounces of urine. Then $455.7 \times .02 \times 40 = 364.5$ grains, or little more than three-fourths of an ounce. Of such a loss Senator well says that half a pound of beef will more than make up the loss of a week.

Other Proteids Found in Urine.

It has long been recognized that modifications of albumin occur in urine, either alone or in association with it, but since the introduction of the delicate tests, more attention has been paid the subject. Among the most constant of these is:

Serum-Globulin, Globulin, or Paraglobulin.—Globulin is almost always associated with serum-albumin, from which it may be separated by Pohl's method as follows:—Render the urine slightly alkaline by ammonium hydrate, and after several hours filter to separate the phosphates. Then add saturated solution of ammonium sulphate in the proportion of one volume to one volume of the filtrate. If a precipitate forms it is globulin.

Globulin is also separated by diluting the urine, after filtration, until the specific gravity is 1003 or 1002. Occasionally a cloudiness appears immediately, due to the separation of some of the globulin. But it is completely separated by passing a stream of carbonic acid through the dilute fluid, for from 2 to 4 hours. In from 24 to 48 hours the globulin falls to the bottom as a milk-white flocculent substance. The supernatant fluid contains the albumin. Should the urine be neutral or alkaline, it must be rendered slightly acid by a few drops of dilute acetic acid.

This test is based upon the fact, that paraglobulin is held in solution by the sodium chloride, and other neutral salts always present in the urine. When urines are largely diluted with moderately pure water, the percentage of neutral salts is so reduced that the globulin falls out of solution.

Dr. Roberts* suggests the following simple modification of the test: Fill a urine-glass or test-tube with water, and let fall into it a succession of drops of albuminous urine. In many cases, each drop as it falls is followed by a milky

* Discussion on Albuminuria before the Glasgow Pathological and Clinical Society, p. 17. Reprinted from the *Glasgow Medical Journal*, 1884.

train, and when a sufficient number of drops has been added, the water assumes throughout an opalescent appearance, as if a few drops of milk had been added to it. The addition of acetic acid causes the opalescence to disappear; for globulin is soluble in concentrated acetic acid, and also in a 1 per cent. solution of hydrochloric acid. From its solution in common salt it is completely separated on heating.

Occurrence.—Not only does globulin accompany serum-albumin in most instances, but, it is said, sometimes exceeds it decidedly, although the proportion in the blood is much less, being to serum-albumin as 1 to 1.5. It may even occur, although very rarely, without serum-albumin. According to Senator, it is most abundant in the urine of lardaceous disease of the kidney. It also occurs in acute nephritis, in the hyperæmia of cantharides poisoning, and in albuminuria associated with deranged digestion.

Mucin.—This proteid, abundant in urine, which has passed over irritated urinary passages, is said to be present, to some extent in all urines. It is not precipitated from solutions by boiling, but is precipitated by alcohol, by dilute mineral acids, and by all vegetable acids except, according to Dr. Johnson, picric acid. But Dr. Oliver does not even except picric acid, and fears that in all of his earlier observations with this acid, he mistook mucin for a trace of albumin.* Dr. Roberts also includes picric acid among the precipitants of mucin.

To Test for Mucin.—The tests usually employed for mucin are citric and acetic acids and by the contact method,

*On Bedside Urine Testing. Third edition, London, 1885, p. 111, note.

the acid being introduced first. It is also precipitated by nitric acid. *Just above* the point of contact a cloud-like coagulum gradually makes its appearance, contrasting with the opaque white coagulum of albumin. When albumin and mucin are both present, the latter appears at the upper part of the column of urine, while the albumin is confined to the point of contact between the two fluids. It differs from the somewhat similar white deposit of acid urates in that it is not dissipated by heat.

Or the following method may be followed:—

To one volume of urine add three of strong alcohol and allow to stand for several hours, when mucin and all albuminous bodies will have precipitated. Filter and wash the precipitate with alcohol, then treat it with warm water. The filtrate, which should contain the mucin, is then strongly acidified with acetic acid, and if turbidity results, it is due to mucin. Or the contact method may be used with acetic or citric acids.

The Mucin Reaction of Normal Urine.—According to Dr. Oliver even when a normal urine is underlaid by citric acid solution, there appears in the course of several minutes, along the plane of contact of the two fluids, a delicate whitish zone which becomes gradually more pronounced, and which is mucin. This reaction, concentrated by the contact method, becomes, when the precipitate is diffused throughout the urine, either totally inappreciable, or appreciable only in the slightest degree. It may be studied by adding a citric acid test-paper to 60 minims of transparent urine. If mucin is present in larger quantity than usual, a slight milkiness appears.

If, however, to the normal urine, acidified by citric acid, a mercuric test-paper be added, a delicate haze may be detected on holding the urine to the light, and on a dark background. This haziness, according to Dr. Oliver, *disappears on applying heat to near the boiling point, and reappears on cooling*, to revanish on reheating. If, on the other hand, a *solution* of the potassio-mercuric iodide is used by the *contact method*,

the opacity thus produced by mucin does not disappear when heat is applied.* If these observations are correct, heat removes all sources of error in testing for albumin with the potassio-mercuric iodide, *when used in the shape of test paper*. This, therefore, Dr. Oliver regards as possessing a distinct clinical advantage over the solution.

Peptone.—The frequent occurrence of peptone in urine has rendered it one of the most important of the possible constituents demanding attention. Much has been lately added to our knowledge of peptonuria, but it is possible that further changes will be made in such knowledge before it is complete.

It is well known that peptone is a proteid substance which is the final result of gastric and pancreatic digestion. It may also be produced from albumin by the continued

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* The student is earnestly recommended to study the reactions of mucin by impregnating normal urine with saliva as directed by Dr. Oliver. The clear saliva and a solution of salt, say 20 grains to the ounce, should be mixed in equal parts, and one drop of acetic acid, or a citric acid test-paper, added to a 4-inch column, which should be thoroughly boiled, when the milkiness produced by a trace of albumin will appear. This highly muciparous solution is added to albumin-free urine, 1 to 1 or 1 to 2 according as the observer may wish to charge it with mucin. In any case the urine will then become more highly muciparous than is likely to be met with in practice. Filtration may be omitted, being slow, if observation is checked by comparing the unheated fluid with that experimented with. A citric acid and a mercuric test-paper, added to 60 minims, produces an opacity exactly like that produced by a small quantity of albumin, *but it differs from it in completely vanishing when heated. The opacity returns as the temperature of the solution falls, and in the cold it greatly exceeds the original amount.* Heat will again disperse it as before. If a trace of albumin be added to the mucin-charged urine, the test papers will produce an opacity which heat will clear up only to a certain degree, that which remains over being due to albumin.

action of acids and alkalies, and it is said, also, by the decomposing action of bacteria, as well as the long-continued operation of a temperature of 268° to 290° F. It differs from albumin and hemialbumose or propeptone in that it is not precipitated by heat or nitric acid, by acetic acid combined with chloride of sodium, or with cyanide of potassium. Like albumin and propeptone, it is precipitated by tannin, corrosive sublimate, phosphortungstic acid, Millon's reagent, and by sodium tungstate, potassio-mercuric iodide and picric acid, the last three being among the recently suggested delicate tests for albumin ; but when precipitated by these reagents it is redissolved by heating. It is further characterized by the purple reaction its solutions strike with sodic hydrate and a little salt of copper. The sensitiveness of this so-called "biuret" reaction is increased by slight colorations of the fluid, and especially a yellow. So delicate is it that a solution as weak as 1 part in 1000 responds promptly. This reaction is shared by propeptone, while albumin produces only a blue coloration—under no circumstances a red or violet. It has been suggested that peptone bears the same relation to albumin as grape sugar to starch—that is, that it is a hydrate of albumin.

Peptone is not present in healthy blood or normal urine, and even during digestion the portal vein contains but traces of it. Injected into the blood, it disappears from it very quickly, a certain proportion reappearing in the urine, while some is taken up by various organs of the body. These organs also appear to retain any small excess which may have been introduced into the blood from over-ingestion of albuminous food. It would seem, therefore, that it is converted into albumin at the moment it is absorbed

into the circulation, and is incapable of taking the place of albumin in the blood, although the reverse has been alleged by Plosz, Maly, and Adamkiewicz. During health, too, the various organs of the body contain traces of it, and it is a constant constituent of pus, as was first shown by Hofmeister.

Tests for Peptone.—The best tests for peptone are those which require some preliminary treatment of urine suspected to contain it. For these we are chiefly indebted to Hofmeister.* The simplest of these is the

Phosphor-Tungstate Test.—(1) The urine is decolorized and freed from mucin by treating say half a liter or about a pint with solution of neutral acetate of lead until a thick flocculent precipitate is produced, and then filtering.

(2) To a portion of the filtrate add acetic acid and a few drops of a solution of ferrocyanide of potassium. Should there be a cloudiness or precipitate it is due to albumin, and the addition should be continued as long as a precipitate occurs. The albumin must be removed by filtration.

(3) Add to a portion of the filtrate about one-fifth its bulk of concentrated acetic acid, and then phosphortungstic acid acidulated with acetic acid.† If the fluid remains clear after standing some time, it does not contain peptone. Should, however, after the lapse of 10 minutes, a cloudiness appear, peptone is present.

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* Zeitschrift für Physiol. Chemie, 573.

† The solution of phosphortungstic acid is made by adding to a boiling watery solution of tungstate of sodium, enough phosphoric acid to produce an acid reaction. After cooling, the fluid is to be made strongly acid with acetic or hydrochloric acid, and, after standing one day, filtered.

The Biuret Test.—Add to the entire filtrate, after treatment with acetate of lead, concentrated solution of tannic acid, so long as a precipitate takes place. Throw the latter, after 24 hours, on a filter, and wash it with water in which tannic acid and magnesium sulphate are dissolved. The precipitate is now thoroughly rubbed up in a capsule with saturated baryta water, and after the addition of a fragment of solid baryta, retained at a boiling temperature for a few minutes. If care is not taken to mix intimately the precipitate with the baryta, resinous masses form during the heating, which interfere with the proper action of the baryta. After a few minutes the mixture is filtered, baryta water again added, and thoroughly shaken with it until the fluid filters off from the dark-colored precipitate, either colorless or slightly yellow.

In the filtrate thus obtained the "biuret" reaction is now sought by adding first liquor sodæ or potassæ until a decided alkalinity is produced, and then, drop by drop, a very weak solution of sulphate of copper. If a reddish color appears, the addition of the copper solution is to be continued until the reddish-violet has reached its greatest intensity. If no peptone is present the fluid becomes simply green or bluish-green. The simultaneous baryta-precipitate does not interfere with the test, as it rapidly subsides while the supernatant fluid retains the color.

Or the lead filtrate may be treated with half a volume or its own bulk of concentrated hydrochloric acid, and then with an acid solution of phosphortungstic acid, until there is no longer a precipitate. The latter is then promptly filtered off, because if allowed to stand, there appears on the surface a second reddish precipitate, which interferes with the subsequent demonstration of the peptone. The

precipitate is washed on the filter with a solution of sulphuric acid, three to five per cent. strong, until the filtrate passes through colorless, then turned into a capsule, intimately mixed with baryta in substance, the mixture treated with a little water, and, after being warmed for a short time, filtered. If too strongly heated, a dark-hued filtrate is obtained, which is to be avoided. To this filtrate the "biuret" test is again applied.

The second method is both shorter and more delicate, detecting, according to Neubauer, peptone in solutions containing but .1 gram to the liter, while the tannic acid method requires .15 to .2 gram. It is the one preferred by v. Jaksch, to whom we are indebted for so much of our knowledge of peptonuria and its clinical significance.

Ralfe's Test.—A rough test for peptone, which answers very well where a considerable quantity is present, may be made by placing a drachm, or 3.5 c.c., of Fehling's solution in the bottom of a test-tube and gently overlaying it with an equal bulk of urine. At the point of contact a zone of phosphates forms, while above this, if peptones are present, a delicate rose-colored halo will float. Should the peptones be mixed with albumin, the halo will be purple.

Randolph's Test with Acid Mercuric Nitrate and Potassium Iodide.—This test, suggested by the late Dr. N. Archer Randolph, of Philadelphia, is based upon the fact, that if Millon's* reagent be added to an aqueous solution of iodide of potassium, a red precipitate of mercuric iodide results, but if peptone or bile acids are present, the precipitate is yellow. To 5 c.c. of urine which must be

* To make Millon's reagent, dissolve one part by weight of quicksilver by the aid of warmth in one part by weight of concentrated nitric acid, and dilute with an equal volume of water.

cold and but faintly acid, add two drops of a saturated solution of iodide of potassium and then 3 or 4 drops of Millon's reagent, when, if peptones or bile acids are present, a yellow precipitate falls. Then the question as to whether it be bile acids or peptones must be settled by the tests for the former.

The Clinical Significance of Peptonuria.—The instances in which peptonuria occurs are numerous. The best determined fact with regard to it is that discovered by Maixner, that it is always present when pus-corpuscles are disintegrating somewhere in the body, and in most of the diseased states in which it has been found, such a condition of affairs has been probable. It has been especially studied by Maixner, v. Jaksch, Fenomenow, and Pacancowski. Among the diseases in which it has been found may be named typhoid fever, variola, scarlatina, miliary tuberculosis, erysipelas, acute arthritis, pulmonary tuberculosis, gangrene of the lungs, croupous pneumonia, purulent pleurisy, embolism, carcinoma of the gastro-intestinal tract and of the liver, catarrhal jaundice, parametritis, cerebral apoplexy, parotitis, abscess.

Exception to the above explanation may have to be made in some cases of carcinoma of the gastro-intestinal tract, and cancer of the liver and uterus. In cancer of the stomach and small intestine, Maixner early ascribed the peptonuria to absorption by the ulcerated surfaces, of the peptone of digestion, but in cancer of the œsophagus, rectum, and uterus, we must have recourse to disintegration of new-formed tissue as the sole source. The almost invariable association of peptonuria with cancer of the liver, Pacancowski thinks, compels the conclusion that the liver in health has something to do with the conversion of

peptone into albumin, an office that in cancer is interfered with.

Although Senator, Petri, and Poehl assert that albuminuria and peptonuria coexist, Maixner, and more recently Pacancowski, fails to confirm this. The latter failed to find it in four cases of chronic nephritis, and one of acute.

It has also been said that peptone may originate from the conversion of albumin by reason of a sort of fermentative action of the cellular elements of urine, and that peptone may be a product of the decomposition of proteid matters by the agency of bacteria.

Hemialbumose, or Propeptone.

This substance is an intermediate product in the conversion of albumin into peptone, during gastric and pancreatic digestion. It is, therefore, abundantly present along with albumin in the gastric and intestinal contents, and, unlike peptone, is also found in the blood during digestion. It was first found by Bence Jones in the urine in a case of osteomalacia, and later by Kühne in another case of the same disease.

Hemialbumose, like albumin, is insoluble in alcohol; sparingly soluble in cold water, but very easily in hot water and water containing only traces of acids, alkalies, or salts. It is not, therefore, precipitated by heat from its watery solution, as is albumin, but if the solution be made strongly acid and concentrated salt solution be added thereto, hemialbumose is precipitated. If the cloudy fluid be now heated, it becomes transparent, but again turbid on cooling. A further large addition of salt maintains the precipitate in spite of heating. Hemialbumose is precipitated by pure nitric acid, but redissolved with the production of an

intense yellow color on being heated, and reprecipitates on cooling. An excess of nitric acid redissolves the precipitate even in the cold, with the production of the same orange-red color.

In these respects hemialbumose differs strikingly from both peptone and albumin. It is like peptone, in that it exhibits the biuret reaction with an alkali and salt of copper. Like albumin, it is precipitated by adding first acetic acid and then ferrocyanide of potassium ; also by phosphormolybdanic, phosphorwolframic, tannic and picric acids, the precipitate, except that with picric acid, being undissolved by warmth.

To test the presence of Hemialbumose, the albumin must first be removed. This is accomplished by acidifying the urine with a few drops of acetic acid, adding about one-sixth its volume of concentrated salt solution, boiling, and filtering off the precipitate. Albumin and globulin remain upon the filter. The filtrate is then allowed to cool, and if a turbidity arises or is produced by the further addition of salt solution, which disappears by heating, and reappears with cold, propeptone is present. If desired, the precipitate can be filtered off the filtrate, redissolved in a little water, and reprecipitated by acetic acid and ferrocyanide of potassium.

Hemialbumose is removed from fluids by adding acetate of iron and boiling, or by boiling with hydrated lead oxide.

Fibrin.

Fibrin is found in the urine when there are hemorrhages from the genito-urinary passages, and in intense inflammation of these passages and of the kidneys ; also in a con-

dition of fibrinuria which occurs in the Isle of France; finally, in chylous urine fibrin is present.

Recognition.—It is recognized by its spontaneous coagulation, the product of which is, however, not to be confounded with mucus, or the glairy substance formed by the action of ammonium carbonate on pus; also by its fibrillar structure as shown by the microscope.

Coagula may be filtered out from urine by means of muslin, and washed with water to free them from urinary constituents. If insoluble in dilute alkalies and in 5 to 10 per cent. solution of sodium chloride, they are fibrin.

The presence of **Pepsin** and **Trypsin** in urine has been lately asserted.

Proteids Contrasted.

The following paragraphs, from the latest edition of Neubauer,* contrasting the characteristic properties of the proteids which may occur in urine, will aid the student in acquiring the necessary knowledge:—

Albumin, hemialbumose and peptone are soluble in water; globulin is not. This insolubility in water globulin shares with protein (which does not occur in urine), while both albuminoid substances are soluble in alkalies and acids, forming with them soluble binary combinations, behaving to the alkalies as acids and to acids as bases. They are soluble also in basic salts (phosphates and carbonates of the alkalies), since they take away from these salts the bases. The combination of protein with a base is called an albuminate, that of protein with an acid, acid-albumin. No special term is applied to the combinations of globulin with bases or with acids.

If the acid be withdrawn from an acid-albumin in solution, by neutralization with an alkali, the insoluble protein is liberated, and falls as a precipitate. The same thing happens when a dissolved albuminate is neutralized by an acid. The term *precipitable albumin* has been suggested for protein, and since globulin, at least under certain circum-

* Wiesbaden, 1881, p. 114.

stances, gives the same reaction, so may the same term be extended to it.

Globulin is distinguished from protein in that it is soluble in neutral salts (the neutrally reacting salts of the alkalies and alkaline earths, as common salt and nitrate of potash); protein is not soluble in these.

Hemialbumose, although soluble in water, is even more easily soluble than globulin in alkalies and acids, as well as in neutral salts. On the other hand, neither alkalies, acids, nor neutral salts have any influence upon the solubility of albumin and peptone, already soluble in water. These two albuminous substances are, however, distinguished from each other, in that albumin is easily converted into precipitable albumin, while peptone is not.

Fibrin, finally, is soluble neither in water, like albumin, hemialbumose, and peptone, nor in dilute cold acids or alkalies, like precipitable albumin, nor in salt solutions like globulin.

Mucin, like precipitable albumin, is not soluble in water, but is in alkalies and strong mineral acids; on the other hand, it is not soluble in dilute mineral acids, nor in concentrated organic acids.

Hæmoglobin and methæmoglobin are recognizable by their color.

VIII. Sugars found in Urine.

Glucose, $C_6H_{12}O_6$.

While the assertion of Brücke and Bence Jones that glucose is present to a slight degree, even in normal urine, has been quite generally accepted, and has apparently been confirmed by the more recent researches of Dr. Pavy,* of London, it has been contradicted by Seegen, and very careful investigations by my colleague, Professor Wormley, confirm the results of Seegen.† Dr. George Johnson,‡ and his son, G. Stillingfleet Johnson, have also lately

* Pavy, Points Connected with Diabetes, London, 1879.

† Seegen, Der Diabetes Mellitus, 2 Aufl., s. 224.

‡ Johnson, G., *Brit. Med. Jour.*, Jan. 8th, 1887.

joined the group who *deny* the presence *of glucose in normal urine.* It does not follow, however, that instances may not occur in which small quantities of sugar, barely, if at all, recognizable by the ordinary tests, are present in urine, and that these have no clinical significance.

Of the large number of tests for the detection of sugar, only those will be given which have borne the trial of experience, or, being new, are so highly commended, that some notice is demanded ; and it is suggested that for practical purposes the student should select some one or more of these and accustom himself to their use, and to the modifications in results to which all are more or less subject. I am confident that much of the difference of opinion as to the value of the different tests is due to the unequal experience of different observers with a particular test. Mistakes are less likely to be made by a beginner with a freshly made Fehling's solution than with Trommer's test. It is necessary, however, to be familiar with more than one test, because cases of doubt constantly arise where the evidence of one is insufficient. (See especially remarks on qualitative testing, p. 100.)

Specific Gravity and Quantity.—The specific gravity alone, when 1030 or more, affords a presumption of the presence of sugar, and if at the same time the urine is very pale, and far exceeds 1500 c.c. (50 fluidounces) in twenty-four hours, the probabilities are much increased. These facts at least call for the use of other tests to determine the question. (See note on p. 25.)*

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* Moore's test, contained in all previous editions of this manual, is intentionally omitted, as superseded by many more delicate tests.

The Copper Tests.

The copper tests depend upon the power which grape-sugar possesses of reducing the oxide of copper and other metallic oxides, as silver, gold, etc., to a lower state of oxidation. In Trommer's test the oxide of copper is set free at the time of its application by liquor potassæ or sodæ, in excess.

Trommer's Test.—1. A drop or two of a (preferably weak—say 1 to 30) solution of cupric sulphate is added to 4 or 5 c.c. of the suspected urine, and then an equal bulk of liquor potassæ or sodæ. On first adding the alkali there is immediately liberated, in addition to the earthy phosphates, a blue precipitate of hydrated cupric protoxide, *which, if sugar is present, is redissolved on agitating the mixture*, producing a beautiful blue transparent liquid. If, on the other hand, there is no sugar, the fluid will not be thus blue after agitation, but exhibit a turbid greenish hue. This, however, is not alone relied upon, but the *mixture is boiled* for a few seconds, and if sugar is present, a copious yellow precipitate of *hydrated* cupric suboxide takes place. This subsequently loses its water and becomes the *red suboxide* which falls to the bottom or sides of the test-tube, to which it often closely adheres.

The precise reaction is not known,

2. A second similarly prepared mixture of these ingredients should be made and set aside without the addition of heat, for from six to twenty-four hours. If sugar is present a similar precipitate of suboxide of copper will take place. If the reaction is at all doubtful it is important that this check-test should be make, since, as Neubauer points out, most of the other organic substances which reduce the salts of copper do so only when heated or after long boiling.

Precautions.—1. Albumin must always be removed, as it interferes with the reduction of the cupric oxide.

2. Too much of the solution of cupric sulphate or too strong a solution should not be used, because the prolonged boiling of any urine with an excess of copper will produce a yellow or greenish-yellow color, which may not appear until the mixture cools off. Just enough copper should be added to produce a distinctly blue color.

3. While the fluid must be made to boil for perhaps half a minute, the precipitate should take place *without prolonged boiling*, as a reduction by other organic substances is induced by prolonged boiling.

4. The flocculent precipitate of earthy phosphates should not be mistaken for the suboxide of copper; it is either transparent or of a pale greenish hue. On the other hand, *a mere change of color is not sufficient*. Strictly normal urine almost always has a *decolorizing* effect. There must be an actual yellow or red precipitate. If it be desired to eliminate altogether any error due to the precipitation of the earthy phosphates, it may be done by adding the potash solution, and filtering before adding the copper.

5. As already stated, cupric protoxide is sometimes reduced by other organic matters found in urine, *especially uric acid*, by hippuric acid, the urates, hypoxanthin, mucus, indican, urochloralic acid found in the urine when chloral is administered internally, turpenoglycuronic acid when turpentine is taken, etc. On the other hand, a small amount of sugar may be present in urine and fail to reduce the oxide in the presence of certain other substances. Dr. Beale* has shown that ammonium chloride, ammonium urate, and other ammoniacal compounds have this latter effect; and not only albumin but organic substances generally, including creatin, creatinin, pepsin, peptones, urinary coloring matters, etc., act similarly. When these partial reductions occur, a yellowish-green precipitate results. Attention should be paid to the specific gravity, to the fact that a precipitate of the phosphates always takes place which must not be mistaken for the suboxide, and the disappearance of the blue color and the substitution of a yellowish tinge is also not to be mistaken for a precipitate. A yellowish *precipitate*, however, does indicate a partial reduction either by some other organic substance

* Kidney Diseases and Urinary Deposits, p. 246.

or by the sugar itself, and demands that the urine should be subjected to the bismuth or fermentation test, or, if absolute accuracy is required, to Brücke's process described on page 84.

When proper precautions are observed, reliable results may be expected with Trommer's test with saccharine urines containing ½ of 1 per cent.

Other Copper Test Solutions—Fehling's, and Pavy's Fluid.

It has been stated that when an alkali is added to a solution of sulphate of copper an abundant precipitate of hydrated cupric protoxide is thrown down. This is not dissolved by any excess of alkali added, but if some organic matter is added or is present, an excess of alkali dissolves the protoxide. It is for this reason that if sugar happens to be present in a suspected fluid to which these have been added, the precipitated protoxide is dissolved and a clear blue fluid results.

These facts enable us to construct a fluid which will hold the protoxide of copper in solution; but, in selecting an organic substance, one must be chosen which will not reduce the oxide of copper as does sugar, else it will make our test inoperative. Such a substance is *tartaric acid*, which is usually employed.

Of the numerous copper solutions employed, only Fehling's, and Dr. Pavy's modifications of it, are given, since these are most convenient in practice, and serve also for quantitative estimation. The one or the other may be used, as it is preferred to work with the English or the metric system.

Fehling's Solution.—34.639 grams pure crystallized sulphate of copper are dissolved in 200 grams distilled water; 173 grams chemically pure crystallized neutral sodic

tartrate are dissolved in 500 or 600 grams solution of caustic soda of specific gravity 1.12, and into this basic solution the copper solution is poured, a little at a time. The clear mixed fluid is diluted to 1 liter.

10 c. c. of this solution will be reduced by .05 gram, or 50 milligrams, of diabetic sugar. If the copper solution is to be kept some time, it is absolutely essential that it should be placed in smaller bottles holding 40–80 grams, sealed, and kept in the cellar. With a view to avoiding the well-known defect of Fehling's solution—its tendency to spoil on keeping—Schmiedeburg suggested the substitution of 15 grams pure mannite for the sodic tartrate. The mannite should first be dissolved in 100 c. c. of water and 500 grams solution caustic soda, sp. gr. 1145, and the solution completed as above. My experience with this solution decidedly confirms Schmiedeburg's statement, and I strongly commend it.

Pavy's Solution consists of—

Cupric sulphate, ...	320 grains.
Neutral potassic tartrate, ...	640 grains.
Caustic potash, ...	1280 grains.
Distilled water, ...	20 fluidounces.

The solution is made in the same manner as Fehling's, and 100 minims correspond to ½ grain of grape-sugar, the formula for grape-sugar being here taken $C_6H_{14}O_7$, while by Fehling it is taken $C_6H_{12}O_6$.*

These solutions serve equally well for qualitative and volumetric testing, but if it is simply desired to have a solution for the former purpose, it may be made by pounding

* This should be remembered, as, in consequence of it, the same urine in the hands of different observers would yield slightly different results, according as one or the other solution is used.

together 5 grains (.324 gram) cupric sulphate, 10 grains (.648 gram) neutral potassic tartrate, and dissolving in 2 drachms (7.4 c. c.) liquor potassæ. The usual blue fluid results.

To Use Fehling's and Pavy's Solutions for Qualitative Testing.—*The same precautions laid down for the use of Trommer's test are to be observed, for the Fehling's and Pavy's solutions are simple modifications of Trommer's test and subject to the same sources of error.*

In using either of the above solutions for qualitative testing, a small quantity, say 1 c. c., should be placed in a test-tube, and *diluted with about four times its bulk of water.* The mixture should then be boiled for a few seconds. If the solution remains clear on this boiling, add immediately the suspected urine drop by drop. If sugar is present in any quantity, the first few drops will usually cause the yellow precipitate ; but the dropping may be continued if no precipitate occurs, reapplying the heat occasionally, until an equal volume of the urine has been added. If no precipitate occurs, sugar is absent, clinically speaking.

If a precipitate occurs on boiling the *test fluid alone*, a new supply must be obtained, or a little more soda or potash may be added, the fluid filtered, when it is again fit for use. The precipitate referred to is a suboxide of copper, the result of a reduction of the protoxide, which sometimes occurs when Fehling's or Pavy's solution is kept for some time. It is said to be due to racemic acid, into which tartaric acid is convertible on exposure. Under the influence of heat this acid oxidizes at the expense of the protoxide of copper, and the suboxide is precipitated ; hence the necessity of boiling a solution, before adding the suspected fluid. I have sometimes noted that boiling a spoiled undi-

luted Fehling solution does not reduce the copper, while from the diluted solution, when boiled, the suboxide is thrown down; so that boiling a *diluted* Fehling's solution becomes a more delicate test of its quality than boiling the undiluted solution. All possibility of such source of error may be avoided by keeping the solution of copper separate from that of the potassic tartrate in the caustic soda solution, and mixing them at the moment they are required for use in the proportion of one part of the former, three of the latter, and two of water.

It will be noted that in the use of Fehling's and Pavy's copper solutions an excess of the test fluid is always used, while in the method described as Trommer's, the effect of adding too much may be to produce a yellow sediment and coloration on boiling with any urine, especially on cooling. It occasionally happens, also, when Fehling's solution is used, that no reaction occurs until considerable urine has been added and the mixture cools down after the boiling, when a yellowness or milkiness makes its appearance. Dr. Roberts* believes this reaction due to sugar. But this is at least doubtful, for I have known it to occur in urine which, when treated by Brücke's process presently to be described, was found to be without a trace of sugar. It may be due to sugar, but it is as likely to be due to uric acid or some other of the reducing substances contained in urine. In such a case the only way to settle the question is to resort to Brücke's process described on p. 84. But this may be said, that the quantity of sugar which could occasion such a reaction is of doubtful clinical significance. Filtering through animal charcoal is a less

* Urinary and Renal Diseases, Amer. ed., 1879, p. 190.

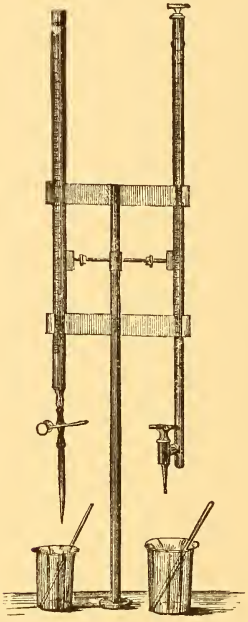
troublesome expedient than the lead process. This gives a perfectly clear fluid to work with, but in such filtration a small quantity of sugar is retained by the charcoal and must be washed out with distilled water. Again, it is almost impossible to obtain charcoal sufficiently free from impurities, even when specially prepared for the purpose.

Quantitative Analysis by Fehling's or Pavy's Solutions. Volumetric Process.—The simplest method of analysis by Fehling's or Pavy's solutions, and one which may be used in the consulting room as easily as the laboratory, is the following: One cubic centimeter of Fehling's solution is diluted in a large test-tube with four cubic centimeters of distilled water, and boiled as described for qualitative testing. Its purity being thus ascertained, $\frac{1}{10}$ cubic centimeter of the suspected urine is added from a suitably graduated pipette. Heat is then reapplied, the precipitate watched, and then another $\frac{1}{10}$ added, the heat again reapplied, until it is found, after proper subsidence, that all the blue color is removed from the cubic centimeter of Fehling's solution. If in doing this 1 c.c. of urine has been added, it will have contained just half of 1 per cent. of sugar. If more than 1 c.c., it will have contained less than a half, but more than one-quarter per cent. If exactly 2 c.c. are used, it will have contained exactly one-quarter per cent. If, on the other hand, but half a cubic centimeter is used, it will have contained 1 per cent., one-quarter of a cubic centimeter, 2 per cent. and so on.

If the proportion of sugar is large, as indicated by the specific gravity or qualitative test, the urine should be diluted with a definite proportion of water, and this regarded in the estimation.

If it is desired to determine the quantity in English measures, Pavy's solution may be used instead of Fehling's, and 100 minims measured off into the test-tube, diluted with four times its bulk of water, and boiled as before. Then the urine, diluted if necessary, is allowed to fall into the liquid, drop by drop, the heat being constantly renewed, until all the blue color has disappeared; and when this has happened the quantity of urine used will have contained just half a grain of sugar.

FIG. 7.



Greater precision may be obtained by the use of the

burette and stand shown in Figure 7. Ten cubic centimeters of Fehling's solution are placed in a porcelain capsule and diluted with 40 c.c. of distilled water. Fill the burette to 50 c.c. with urine diluted from 5 to 20 times, according to the proportion of sugar estimated by a preliminary trial. The capsule containing the diluted Fehling's solution should be placed on a wire gauze and heated with a gas flame or spirit lamp, when half a c.c. of urine is allowed to fall into the hot solution from the burette. Immediately a yellow or red precipitate will fall. This is allowed to subside, and if any blue color remains the urine is cautiously added, the solution being kept hot, until all the blue color disappears. The titration must be repeated, if necessary, until the exact point of disappearance is ascertained and noted. If 5 c.c. of the urine are used to decolorize 10 c.c. of Fehling's solution, since 10 c.c. of Fehling's solution corresponds to .05 gram of sugar, the 5 c.c. of urine will have contained exactly this quantity, that is, .05 gram. If now the urine has been diluted 10 times, the 5 c.c. will contain .5 gram sugar, and 100 c.c. will contain 10 grams or 10%. Pavy's solution may be used similarly.

Cupric Test Pellets.—These were first suggested by Dr. Pavy at a meeting of the Clinical Society of London, in January, 1880, and were first made in this country by Mr. McKelway, of Philadelphia, at the suggestion of Dr. Joseph Neff.* They contain the elements of Fehling's solution in solid form, and are very neatly made in the shape of a compressed pill. As made by Mr. McKel-

* New York Medical Record, March 23, 1880.

way, each pellet when dissolved in distilled water represents 5 milligrams of diabetic sugar, and they may be used for approximate quantitative as well as qualitative testing.

To use them, dissolve one in a small quantity of water in a test-tube and boil the solution. The purity of the pellet is thus tested, for if it has spoiled, the cupric oxide is reduced spontaneously. If the solution remains clear, the urine is added drop by drop, the temperature being kept up, and if sugar is present the usual precipitate occurs. The quantity of urine which just removes the blue color of the solution in which one pellet is dissolved contains 5 milligrams of sugar.

These pellets are very convenient to carry, but in my experience they are liable to become unfit for use sooner than a Fehling's solution, and unless very carefully made they keep but a short time.

Pavy's Ammoniated Cupric Test.—Dr. Pavy has suggested, in lieu of the usual form of Fehling's Solution, an *ammoniated* cupric test solution, which has the advantage of obviating the precipitate of suboxide of copper, which is held in solution by the ammonia, so that a simple decolorization takes place as the cupric oxide is reduced; the completed reduction being indicated by the total disappearance of the blue color.

The proportions are as follows:—

METRIC SYSTEM.

Cupric sulphate	4.158 grams.
Potassic sodic tartrate,	20.400 "
Potash caustic,	20.400 "
Strong ammonia (Sp. gr. 0.880),	300 c.c.
Water to one liter.	

ENGLISH SYSTEM.

Cupric sulphate,	36½ grains.
Potassic sodic tartrate,	178 "
Potash caustic,	178 "
Strong ammonia (Sp. gr. 0.880),	6 fluid oz.
Water to one pint.	

Dissolve the potassic sodic tartrate and potash together in a portion of the water, and the cupric sulphate with the aid of heat in another portion; pour the solution of cupric sulphate into the mixture of potassic sodic tartrate and potash; when cold add the ammonia and, finally, with water, bring the volume of liquid to the bulk specified.

Ten c.c. of the liquid is a convenient quantity to work with and does not call for the employment of any appliance to obviate the inconvenience arising from the evolved ammonia. In performing the analysis twice the volume of water is added to the 10 c.c. of test employed.

It should be stated that Hehner, Yeshida and Sutton assert that by this method the ratio of reduction is seriously influenced by the amount of fixed alkali present and by the strength of the ammonia.

Cupric Test Paper.—Dr. Oliver has also constructed a cupric test-paper with tartrate of cuprammonium, this salt being selected as the only one which is permanent on exposure to the air, and the only one that is stable when boiled with an alkaline carbonate as well as with a caustic alkali.

The test-paper is a compound one, consisting of one charged with the reagent and the other with carbonate of sodium, the two being united by a thin layer of rubber.

To use.—1. Drop a test-paper into 60 minims of soft or distilled water. 2. Boil for a few seconds until the water assumes a greenish tint. 3. Remove the papers. 4. Reboil the solution, and then add one drop of the suspected urine. 5. If glucose be present, reduction will take place without further application of heat, though, if preferred, the boiling may be continued and the reaction hastened. In any case, if the solution remains transparent for a quarter of a minute, heat should be applied to the boiling point for one minute. If then no opacity whatever appears, it may be safely inferred that glucose is not present in pathological amount.

The Fermentation Test.

An excellent test for the presence of sugar is the fermentation test. The most convenient method of its application is as follows: A small quantity of ordinary baker's or brewer's yeast (about a fluidrachm, or 3 to 4 c.c.) is added to about 4 ounces of urine in a 6-ounce vial, which is lightly corked and subjected to a temperature of 15–25° C. (59–77° F.). If sugar is present evidences of fermentation will present themselves generally within twelve hours, by the formation of carbonic acid gas, which passes off, leaving the fluid lighter and reduced in specific gravity in proportion to the quantity of sugar present.

Dr. Roberts early announced that urine containing less than 0.5 per cent. or 2½ grains to the ounce, yields no sign to the fermentation test. Claims to much greater delicacy are made, but it is evidently impossible to discover a much smaller proportion, because water will absorb an equal bulk of carbon dioxide, so that all the gas generated in a solution containing $\frac{4}{10}$ per cent. must disappear.

Dr. Roberts has made use of the fact of the lowering of the specific gravity in devising a **quantitative method.** He has shown by careful experiments that every "degree" in specific gravity lost in fermentation corresponds to *one grain of sugar per fluidounce.* Thus, if before fermentation the specific gravity of a given specimen is 1050, and after fermentation it is 1020, it will have contained 30 grains to the fluidounce. The method recommended by Dr. Roberts is as follows: Four ounces of the saccharine urine are put in a 12-ounce bottle, and a piece of compressed yeast, as large as a small walnut, is added. The bottle is then covered with a nicked cork to permit the escape of

the carbonic acid, and set aside on a mantlepiece or other warm place. Beside it is placed a tightly-corked 4-ounce vial, filled with the same urine, but without any yeast. In eighteen to twenty-four hours fermentation will be complete, and the scum cleared off or subsided. The specific gravity of the decanted fermented urine is then taken; at the same time, that of the unfermented urine, and a comparison made. While some time is required to complete the fermentation, yet, as Dr. Roberts says, the preparation can be made by the patient himself or friends, and each day, when the physician makes his visit, he has only to make the comparison.

The percentage may be roughly arrived at by multiplying the number of degrees lost by .23.

Appliances have been suggested within the past year by Max Einhorn and S. P. Kramer, with the object of securing greater accuracy in the quantitative estimation by fermentation; but, as the results are at best but approximate and the apparatus even less convenient than that suggested by Roberts, I omit them. They may be purchased with directions for their use from Einer & Amend, New York City.

Böttger's Bismuth Test.

This consists in adding to urine an equal volume of liquor potassæ or sodæ, then a pinch of the ordinary subnitrate of bismuth, shaking and boiling for a couple of minutes. The sugar possesses the power of reducing the salts of bismuth, and, if present, the black metallic bismuth will shortly be deposited on the side of the test-tube. If the quantity of sugar is small, the bismuth will assume a grayish hue; hence, when this is the case, a very small amount of bismuth should be used in making the test.

This is a brilliant test, and except albumin or other substance containing sulphur, nothing but sugar is supposed to reduce bismuth salts. To meet this, especial care must be taken to remove the albumin before applying the bismuth test, or we may use

Brücke's Modification of the Bismuth Test.*— Professor Brücke finds that while Böttger's bismuth test has many advantages over Trommer's test, it may lead, under certain conditions, to false results, since sulphur occasionally present in the urine will cause a black precipitate of sulphide of bismuth; hence he recommends the use of Frohn's† reagent to remove the disturbing elements, as follows: take equal quantities of water and urine in two test-tubes; to the first add hydrochloric acid until a drop of the Frohn's reagent no longer produces a cloudiness. In this way we ascertain approximately how much HCl must be added to the urine. Acidify the urine with such quantity; treat it with the reagent and filter. The filtrate, which should not now become cloudy on adding HCl or the reagent, is boiled for a few minutes with an excess of a concentrated solution of caustic soda or potash, as in Böttger's test; if a gray or black color results, or such a precipitate is formed, the presence of sugar is proven beyond a doubt.

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* Proceedings of American Pharmaceutical Association, 1877, p. 287. Also Hoffmann and Ultzmann, Analysis of Urine, American translation. New York, 1879, p. 93.

† 1.5 gram freshly precipitated basic bismuth nitrate is mixed with 20 grams water and heated to boiling; then 7 grams iodide of potassium and 20 drops of hydrochloric acid are added. The reagent is orange red.

The Picric Acid and Potash Test.

This test, originally suggested in 1865 by C. D. Braun,* and revived by Dr. George Johnson† in 1882, is based upon the fact that grape-sugar, when boiled with picric acid and potash, reduces the yellow picric acid to the deep red picramic acid, the depth of color depending upon the amount of sugar present.

For qualitative testing, to a fluidrachm of the suspected urine, add 40 minims of a saturated solution of picric acid, and half a drachm of liquor potassæ‡. If albumin is present, a turbidity will develop on the addition of picric acid, and thus the presence of this substance be recognized; but it does not interfere with the test. Boil the mixture, and if sugar is present, a dark mahogany-red color will be produced. If normal urine be treated in the same way, a somewhat darker hue is also developed, but not nearly so marked as when sugar is present.

Or the test may be applied, especially at the bedside, in the following manner:

Into a test-tube, graduated up to three drachms, is put about one-third of a grain of picric acid—as much as can be carried on the point of a penknife; then add half a drachm of water; the acid is dissolved in the water by the

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* Ueber die Umwandlung der Pikrinsäure in Pikraminsäure, und über die Nachweisung der Trauben-Zucker.—*Zeitschrift für Chimie*, 1865.

† Lancet, November 18th, 1882.

‡ Dr. Charles F. Adams has altered the strength of the solutions so that the same result is obtained by taking 5 c.c. of each. Thus, of urine 5 c.c.; liq. potassæ, sp. gr. 1.036, 5 c.c.; sol. picric acid (gr. 3.5 to f℥) 5 c.c.; water 5 c.c.

heat of a lamp. Now, add half a drachm of urine, and the presence of albumin is ascertained; next add a grain lump of caustic potash, and boil the liquid for a few seconds, and the dark coloration appears.

For quantitative testing Dr. Johnson directs a standard solution made by boiling together a fluidrachm of a solution of grape-sugar of the strength of one grain to the fluidounce, half a drachm of liquor potassæ, and forty minims of a saturated solution of picric acid, the whole increased to four drachms with distilled water. The mixture is conveniently made in a large test-tube, which should be marked at four drachms. The liquid is kept boiling for sixty seconds, during which its pale-yellow becomes a beautiful claret-red. It is cooled by cautiously immersing the tube in cold water, and if the level of the liquid is not that of the four-drachm mark, it is raised to it by adding distilled water. The color, thus obtained, is that which results from the decomposition of 40 minims of a saturated solution of picric acid by a grain of sugar to the ounce, four times diluted. The color of this solution is, however, not permanent, and Dr. Johnson imitates it with a solution of ferric acetate made by mixing thoroughly ʒj of solution of perchloride of iron, sp. gr. 1.44; ʒiv of solution of acetate of ammonium; ʒiv of glacial acetic acid, sp. gr. 1065; adding ʒj of liquor ammoniæ, and diluting with distilled water up to f ʒiv. The ingredients are all of the strength of the British Pharmacopœia.*

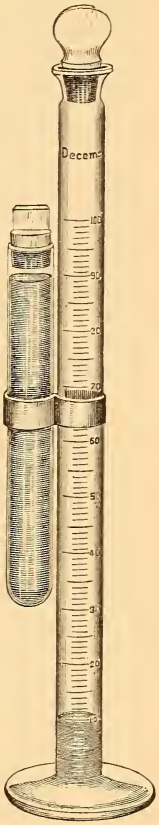
* Dr. Adams has also substituted the ingredients by those of the United States Pharmacopœia, much more convenient to the American worker, as follows:—

Liquor ferri perchlor. f ʒj; Liq. ammon. acetat. f ʒss; Ac. acet. glacial. f ʒss; Liq. ammon. f ʒj; Aqu. destil. ad f ʒiv.

This solution, corresponding to a grain of sugar to the ounce, four times diluted, retains its color unchanged, at least when kept in the dark, and is used for comparison.

In testing for sugar, treat a fluidrachm of urine as indicated for qualitative testing, and dilute the mixture with distilled water, up to four fluidrachms. The test-tube, which should be a large one, may be conveniently marked at four fluidrachms. Raise the liquid to the boiling point and keep it boiling for sixty seconds. Cool the liquid by carefully immersing in cold water, and if below the four-drachm mark it is to be again raised to it by the addition of distilled water. A comparison is then made between the resulting fluid and the standard solution by means of the picro-saccharometer devised by Dr. Johnson and illustrated by Figure 8. The stoppered tube on the left contains the standard solution. Into the graduated tube on the right, having the same diameter as the standard tube, the boiled mixture is to be introduced. Should the two agree precisely in color, the suspected urine will have contained one grain of sugar to the ounce. If, on the other hand, the mixture requires to be diluted in order to make it correspond with the standard solution, this should be done with distilled water, and if enough must be added to raise the level of the

FIG. 8.



fluid, say from the 10-division mark, at which it stood, to the 20-mark, the quantity of sugar would be two grains; if to 40, four grains; to 45, four and a half grains.*

In making an analysis, the picric acid must be added in proportion to the amount of sugar. If the proportion of sugar be as high as six grains per ounce, a drachm of the picric acid solution will be required. If it is higher than this, the urine should be diluted with distilled water in a definite proportion before commencing the analysis, and the dilution borne in mind in making the calculation. When the urine has been diluted ten times, the figures on the saccharometer indicate the number of grains per ounce. Thus, when the ten-times diluted urine, after boiling with picric acid and potash, is further diluted from 10 divisions to 35, to obtain the standard color, the amount of sugar is 35 grains to the ounce.† We may reduce the amount of sugar per ounce to the proportion per cent. by a simple proportion, in which the first term is 455.7 grs., the weight

* A more exact comparison of the saccharine liquid with the standard can be made by pouring into a flat-bottomed colorless tube, about six inches long and an inch in diameter, as much of the standard as will form a column of liquid about an inch in height, and an exactly equal column of the saccharine liquid in a precisely similar tube. Looking down on the surface of a white porcelain slab or white paper through both tubes at once, a slight difference in tint is easily recognized; and if the liquid to be analyzed be found darker than the standard, it is returned to the saccharometer and diluted until the two fluids are found to be identical, when the final reading is made.

† Distilled or clear rain-water should be used, because the turbidity resulting from the action of potash on the salts of lime interferes with the exact estimation of the depth of color.

of an ounce of water at 0° Cent.,* 100 the second term, and the quantity of sugar per ounce the third. Thus, if there be 20 grs. of sugar to the ounce, then as 455.7 : 100 : : 20 : 4.3.

It has been said that when normal urine is treated with picric acid, potash, and heat, a slight coloration also takes place, which Dr. Johnson estimates as about equal to the change which would be produced by a solution of glucose containing 0.5 to 0.7 grains to the fluidounce. Dr. Johnson, in common with others, now ascribes this reaction to creatinin.

Dr. Johnson, after much experimental research made in association with his son, G. Stillingfleet Johnson, claims that this method is "as accurate as any other," and that for the estimation of sugar in the urine it is even more accurate than either Fehling's or Pavy's process, because the picric acid is not acted on by uric acid or urates, which reduce the oxide of copper. He also claims that the method by the picro-saccharometer is more speedy than any other, the materials and apparatus inexpensive, and not liable to undergo rapid change.

The Indigo-carmine Test.

When a solution of indigo-carmine alkalized by sodium carbonate, is boiled and kept heated, the blue color remains, but when a drop of a solution of glucose or saccharine urine is added to the hot solution, a beautiful play of colors occurs, terminating in pale yellow. Mulder first

* This is the weight of the ounce of distilled water according to the United States Pharmacopœia. The English fluidounce weighs 437.5 grains.

suggested this reaction as a test for sugar. Dr. Oliver has revived it for both qualitative and quantitative analysis. The indigo-carmine solution is not available, because when the carmine and sodium carbonate are present in solution the fluid undergoes a gradual change, the indigo-blue color gradually passing into a pale green. Nor is it convenient to keep the solutions separate.

Dr. Oliver has, however, constructed a stable test-paper, charged with a definite quantity of the two reagents, indigo-carmine and sodium carbonate ; while increased sensitiveness is produced by the use of an additional paper charged with sodium carbonate.

In Testing.—1. Unless very recently prepared, one of the carmine test-papers should first be gently heated, say on a knife blade or spatula, by being held for a few seconds just above a flame.* It should then be dropped into a half-inch test-tube, and water should be poured in to the amount of 60 minims.

2. Heat is applied, the tube being gently shaken, and boiling kept up for a second or two. The solution will then be quite blue, and, if the water added was soft or distilled, it will be perfectly transparent. Any turbidity observed will arise from the use of hard water, in which case a sodium-carbonate paper should be dropped into the solution. The test-paper may now be removed or allowed to remain.

3. Not more than one drop of the suspected urine is let fall into the tube from the pipette held in an upright position.

* This is necessary, because the test-paper, on being kept for some time, loses its sensitiveness for detecting small quantities of sugar, say 1 to 2 grs. per ounce.

4. The contents of the tube are again boiled for a few seconds; then the tube should be raised an inch or two above the flame, and held without shaking while the solution is kept quite hot, but without ebullition, for exactly one minute. If glucose is present "in abnormal amount," says Dr. Oliver, "the soft rich blue will be seen, first of all, to darken into violet; then, according to the quantity of sugar, there will appear in succession purple, red, reddish-yellow, and finally straw-yellow." When the last color has developed, agitation will cause the return of purple and violet, and finally of the original blue.

The time required for the reaction to commence, after boiling, varies inversely with the amount of glucose present. When the latter is large—over 20 grs. to the ounce—it will amount only to a few seconds. When small—say from 2 to 3 grs. to the ounce—it may require from 30 to 60 seconds. If the urine contains less than ½ a grain to the ounce, the color of the solution at the end of one minute will be unchanged.

Precautions.—1. Care should be taken during the heating not to shake the tube, or to keep up active ebullition.

2. While keeping the contents of the tube hot, it should not be held between the eye and the sky, for then the early color changes may escape detection. The tube should be kept below the eye level, and its contents viewed by the reflected light of some bright object—as a sheet of white paper propped up an inch or two beyond the tube.

The test is as available by artificial as by daylight.

3. Any caustic alkali, as liquor potassæ or sodæ, will discharge the blue color of carmine. Hence, care should be taken not to use a test-tube containing a trace of either of these substances or of Fehling's solution or the alkaline picric solution, lest a deceptive reaction occurs. No amount of agitation will restore the blue removed by these agents.

Dr. Oliver, comparing the results of experiments with the

indigo-carmine and Fehling's solution, found that whenever one drop was submitted to the indigo test and the presence of sugar shown, confirmation was invariably provided by Fehling's solution used in the ordinary way. On the other hand, whenever one drop of urine gave no reaction with the indigo, Fehling's solution also gave negative results.

In further experimental testings, Dr. Oliver found that none of the ordinary constituents of urine affect the carmine test, but all the free acids of the urine, uric, oxalic, lactic, etc., reduce Fehling's solution. Of substances apt to appear in urine in disease, albumin, peptone,* pus, mucus, blood, bile, leucin, tyrosin do not react with either test; nor does one drop of ammoniacal or decomposing albuminous urine, or weak solution of ammonium sulphide ;† but dextrin and milk-sugar as well as glucose reduce both. Inosit reacts with the carmine, and turns Fehling's solution green—a green precipitate falling, leaving the supernatant fluid blue, which, however, becomes green on reheating.

Of medicinal agents likely to find their way into the urine, iron sulphate, gallic and tannic acids alone react with carmine, as do they, also, with Fehling's solution.

Comparative experiments by Oliver with picric acid and potash showed that whenever the indigo-carmine test-paper afforded a reaction, a correspondent reaction was obtained with the picric solution.

Dr. Oliver has also applied his test to quantitative analy-

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* In a letter from Parke, Davis & Co., I am informed that peptone decolorizes the solution just as does iodized starch.

† Even a weak solution of ammonium sulphide will reduce Fehling's solution.

sis, but it is rather cumbersome and not likely to be availed of. It is therefore omitted from this edition.

The Phenyl Hydrazin Test.—This test, suggested by Emil Fischer, is of such recent origin that its true value has not been determined; although it is claimed for it that it is exceedingly delicate. The following directions for its preparation are given by Prof. T. C. Van Nüys, in his recent excellent book on chemical analysis. To 50 c.c. (13.5 f℥) of the suspected urine add 2 grms. (30.8 grains) of phenyl hydrazin hydrochlorate, and 1.5 grm. (23 grains) sodium acetate or 1 grm. (15.4 grains) of the latter, if the urine is not decidedly acid. Add also 20 c.c. (5.4 f℥) of water unless the urine is nearly colorless. The capsule or beaker containing the urine and reagents is then placed in a water-bath and gently warmed for one hour. If sugar is present, needle-shaped crystals of phenyl glucosazon will have appeared, which must be recognized by the microscope. But this is not sufficient. Their nature must be confirmed by determining their melting point which is 204 to 205° C. For this purpose the dry crystals are obtained by filtering them off the urine, washing with a small quantity of water, dissolving in a small amount of dilute alcohol and recrystallizing by evaporating at a low temperature. After repeating this two or three times the crystals are collected in a desiccator over sulphuric acid.

Then draw out a piece of thin glass tubing in a Bunsen or spirit lamp flame, so that the sealed extremity is 2 or 3 c.m. (.8 or 1.2 in) from where the tube is of original diameter. The tube is broken by a file near where the contraction begins, and a small quantity of the dry body is introduced into the sealed extremity. The piece of tubing is now attached to a thermometer by a small rubber band. The capillary end of the tube containing the substance is placed adjacent to the bulb of the thermometer, and the tube with the bulb is placed into concentrated sulphuric acid in a beaker which is gradually heated. As the mercury ascends to 204° C., the substance will begin to show evidence of fusion, providing the increase in temperature be gradual and the heat be equally diffused by stirring the acid with a glass rod.

Dr. A. K. Bond, of Baltimore, publishes* the following simple modification of this test, suggested by Ultzmann, of Vienna.

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* Medical News, Aug. 6, 1887.

Pour the phenyl salt into a test tube to the depth of about $\frac{4}{10}$ of an nch, and add crystals of sodium acetate, ground fine, to an equal height. Upon this pour the urine—clear or cloudy—until the tube is half full. This gives in a test-tube five inches long, about the following proportions in weight: 1 part phenyl salt, 2 parts sodium acetate, 15 parts urine. Shake the tube until the crystals of sodium acetate are dissolved; heat gently over a low flame until the mixture boils, and boil for about half a minute—whether it becomes clear or not makes no difference. Then cover the tube and let it stand, and, after a proper interval, examine the sediment with the microscope. If sugar is present, there will be seen first fine, bright yellow needles, which branch out or are joined by others as they are formed, until the field is dotted with groups like delicate sprays or sheaves, or radiating from a centre. A magnifying power of 200 diameters is sufficient for this study. That the phenyl salt is always in excess when the test is made in this way is shown by the constant presence of reddish globules in the field.

The Alpha Naphthol and Thymol Test. This recent test for sugar as directed by Molisch is made by adding to 1 c.c. of the fluid to be tested 2 drops of a 15 to 20% solution of alphanaphthol, and after mixing, an excess of concentrated sulphuric acid. Upon shaking, if sugar is present, a deep violet color is developed, and on dilution with water a violet blue precipitate occurs soluble in alcohol and ether with a yellow color, and in potassium hydrate with a deep yellow. If the alphanaphthol is replaced with thymol a deep red color is produced, and on dilution with water a carmine red flocculent precipitate, soluble with a pale yellow color in alcohol, ether and potassium hydrate, but with a bright yellow in ammonium hydrate. These tests, according to Molisch, are the most delicate known, detecting sugar in solutions containing .0001%.

It is claimed that most sugars, to which inosit is an exception, respond to these tests, as do most glucosides except indican. Urea, creatinin, xanthin, uric acid, allantoin, hippuric and succinic acids, phenol and pyrocatechin all give negative results. Normal urine, however, responds when diluted 300 times, from which Molisch concludes it contains sugar. He gives the following method of distinguishing normal from diabetic urine. 1. Dilute a specimen of normal urine and one of the urine to be tested for sugar with 100 volumes of water, and compare the colors resulting from the application of the tests. 2. Dilute two similar speci-

mens with 300 or 400 volumes of water. The diabetic urine will still respond to the test while normal urine fails.

Seegen has carefully examined these tests and declares them less sensitive than Trommer's, while various animal substances and secretions and chemically pure albuminous bodies, as peptone, serum albumin, egg albumin, and casein, all reacted. Hence he says they are of no value as tests for sugar alone, and Molisch's conclusion that glucose is a constituent of normal urine is not justified.

In reply to Seegen, Molisch reasserts his original claim, and says that in dilute solutions it is necessary to employ a small quantity of *solid* alphanaphthol in place of naphthol *solution*. With reference to albuminous bodies, Molisch says that while these may give results resembling somewhat those obtained with sugar solutions, still the precipitates obtained upon dilution with water are, excepting in the case of peptone, all of a different color (dirty yellow or yellowish brown) from those produced with sugar solutions. Besides, they are all soluble in hydrochloric acid with a carmine-red or reddish-violet color, while the precipitate obtained with sugar solutious is insoluble in hydrochloric acid.

Molisch also states that in place of sulphuric acid, hydrochloric acid may be employed in these tests, the mixture being subsequently boiled for a minute.

Fibrine, vitellin, serum albumin, egg albumin and peptone do not give the reaction when hydrochloric acid is used. Normal urine gives the reaction when boiled with alphanaphthol and hydrochloric acid even if diluted ten times. Molisch still asserts that normal urine must contain sugar, or otherwise some body as yet unknown.

Polarimetry.

The most convenient and the quickest of the methods for the quantitative determination of glucose, when the quantity exceeds 1%, and when all the appliances are at hand, is by polarimetry. Smaller amounts can of course be detected,—it is said .01 of 1 per cent. with the best instruments by those who are expert. The costliness of the apparatus, however, will probably always be in the way of its general use.

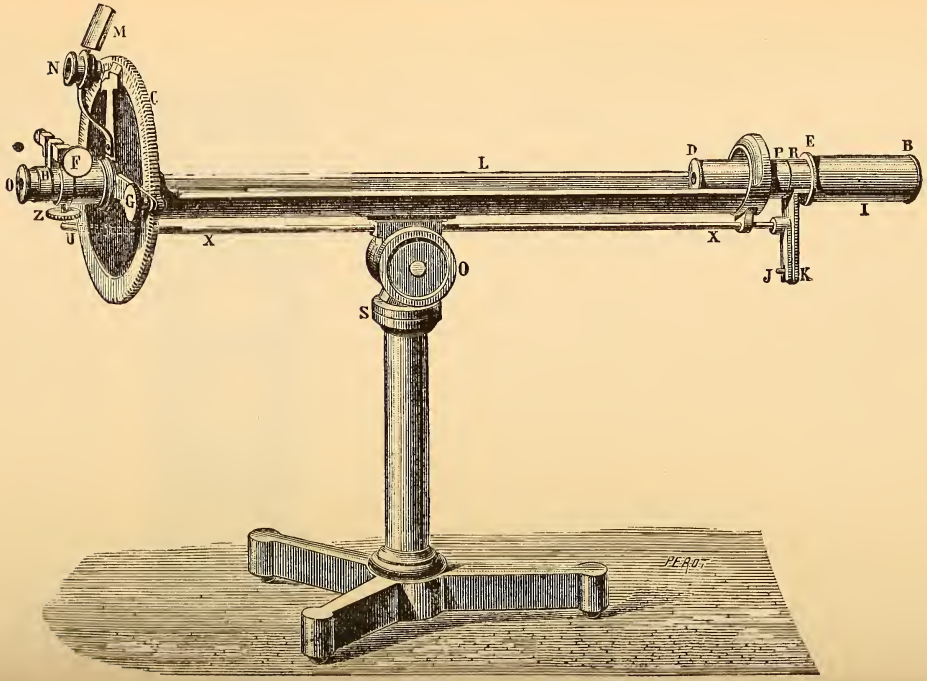
The process is based upon the fact that glucose rotates polarized light toward the right, and the proportion of sugar in solution is determined by the degree of deviation noted. The best instrument probably is the shadow apparatus of Laurent, shown in Figure 9. Light is admitted through a Nichol's prism or polarizer in the tube RB, and falls on another prism, the analyzer in the eyepiece OH.

A ray of light entering the polarizer is divided into two parts vibrating at right angles to each other, but the construction of the prism is such that the ray vibrating in one plane is absorbed while the other passes through. The latter is therefore polarized. Now if the second prism or analyzer in the eye-piece OH is so placed in relation to the polarizer that their oblique ends are parallel, the polarized ray will pass through the analyzer without obstruction. If, however, the analyzer be rotated on its axis, the light gradually diminishes until the rotation reaches 90°, when it is totally cut off. After 90° is passed, the light begins to return in increased quantity until 180° is reached, when it again passes unobstructed. Continuing the rotation, it is again gradually diminished until 270° is reached, when it is again totally absent. After 270 it again returns until 360° degrees, or the starting point, is reached.

Now if between the prisms so arranged that light passes through unobstructed a column of glucose is placed, the plane of vibration of light is rotated by the sugar so that when it reaches the second prism it is partly obstructed. By turning this prism on its axis the light may again be transmitted. The quantity of sugar and the length of the column determine the degree of deviation. Hence, knowing the specific rotary power of glucose, and the length of the tube, together with the angle of deviation, it is easy to calculate the percentage of sugar. If the tube is 10 c.m. long, we have simply to multiply the degrees of rotation by 100 and divide by 53.1, the specific rotation of diabetic sugar.

In most polarizing saccharometers the analyzing prism is attached either to the graduated arc of the circle or to the vernier, so that when one or the other is turned the prism is turned. But the difficulty of determining the precise moment at which the maximum quantity of light

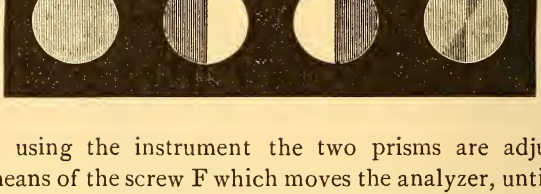
FIG. 9.



The Laurent Shadow Polarizing Saccharometer.

is passing through is so great that some special means is provided for recognizing it. In Laurent's so-called "shadow" instrument, a quartz plate is so interposed that when the prisms are placed in certain relation, half of the field of view is dark and the other light; in other positions both halves are equally light, while in intermediate relationships intermediate shades appear, as in Figure 10.

Fig. 10.



In using the instrument the two prisms are adjusted by means of the screw F which moves the analyzer, until the field is uniformly illuminated on both sides. The vernier should then read 0. The suspected urine, freed from albumin, if this should be present, is treated with a solution of basic acetate of lead in the proportion of 1 to 10 of urine, and filtered. The tube of known length is then filled with the mixture, and placed in position between the two prisms. Immediately light passes through the analyzer in OH, and this must be again turned by means of the screw F until the light is again stopped and the field is uniformly illuminated. The angle is now read off on the dial-plate C and the percentage calculated—if the tube is 10 cm. long, by multiplying the degrees of rotation by 100 and dividing by 53.1, the specific rotation of diabetic sugar. To this result should be added $\frac{1}{10}$ to allow for the 10 c.c. of lead solution* used.

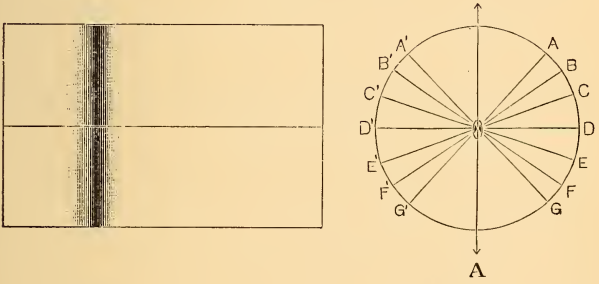
Until recently a sodium flame has been the source of

* The urine may also be clarified by filtering through animal charcoal, in which event, of course, no allowance of this kind need be made.

light, but Laurent has further improved his apparatus, so that an ordinary gas-flame may be used. A new vernier is interposed between the analyzer in OH and the dial-plate C, Fig. 9, and we have simply to multiply the reading increased by $\frac{1}{10}$ (allowance for the lead solution) by the figures .2051, a factor arrived at by experiment as that which multiplied by the reading gives the percentage when a tube 200 millimetres long is used. Thus, suppose the reading to be 24.9, then $24.9 + 2.49 = 27.39$, and $27.39 \times .2051 = 5.61$ per cent.

In the instrument of von Fleischel, in use by the writer, and with which a condensed petroleum flame is used, when adjusted and the vernier reads 0, two spectra are placed one above the other, with the colors in each exactly continuous, the dark band proceeding through the centre of both, as shown in Fig. 11.

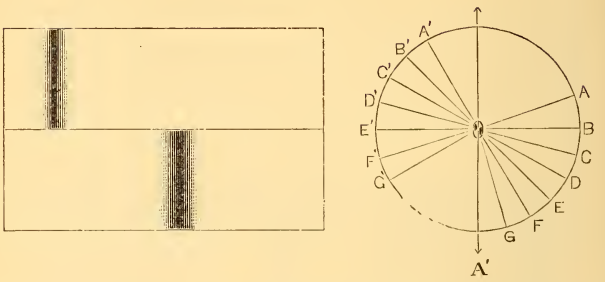
Fig. 11.



When the column of sugar is interposed the relationship of the spectra is at once altered, as shown in Fig. 12.

The proper relationship is again restored by moving the

Fig. 12.



vernier to which the analyzing prism is attached, and the *percentage* of glucose is at once read off.

For qualitative testing the polarizing saccharometer is less convenient than Fehling's solution, indeed will not detect as small quantities. Its greatest convenience appears in quantitative determinations where quantities of over 1½ per cent. are to be measured. This instrument is less costly than Laurent's.

Remarks on the Qualitative Testing of Urine suspected to contain Sugar.

There has been more criticism of the methods employed in testing urine for sugar, and the results obtained from them, than is justified. It arises partly from the fact that the chemist and the clinical physician view the subject from different standpoints. The former makes his experiments with test-fluids upon pure aqueous solutions of sugar, the latter upon a fluid containing numerous other organic constituents, more than one of which is capable of influencing certain test-solutions, though it may be in a less degree than

sugar. Let us suppose the chemist to have made solutions of grape-sugar of different strengths which he is testing with Fehling's solution diluted in the manner indicated on p. 75. He tests weaker and weaker solutions, and finally reaches one by which the test-fluid is just decolorized, while pure water will have no such effect. The chemist knows that it is sugar which thus reacts, because he has nothing but the test-fluid, sugar, and water in combination. But it is very different with the clinician who tests urine suspected to contain sugar. There is no urine which will not partially decolorize a Fehling's solution sufficiently dilute, when it is boiled with it. Yet no one can claim that it is sugar which produces the decoloration when there may be several organic substances in the urine which can do it. On the other hand, no one can deny that it is sugar, because a very small amount of sugar will do the same thing. Further, it is easy to show that a quantity of sugar so small that it cannot be detected in urine can be readily detected in water, though it is also true that minute traces of sugar can be extracted from urine by certain processes, one of which will be detailed below. But these processes are not often available for the practical physician, nor is it necessary that they should be. For such amounts of sugar have no more clinical significance than has the normal proportion of urea in a specimen. It is only tangible amounts of sugar which are significant, and these are ordinarily recognizable by any one of the tests named, *practiced with due precaution.* With all tests, there are certain very evident reactions and certain doubtful ones, and to interpret these last, experience must often be relied upon.

Exact Qualitative Method of Testing for Glucose—Brücke's Lead Process.

While any one of the methods just detailed suffices, if carefully used, to determine qualitatively, with sufficient accuracy for clinical purposes, the presence of sugar in a given specimen of suspected urine, it sometimes happens, especially in watching the course of a case of diabetes mellitus under treatment, that the physician desires to know with absolute certainty whether there is even a trace of sugar present. This can be done by the following method, which is a modification of the one originally proposed by Brücke, and used by Pavy to demonstrate the presence of sugar in normal urine:

Take 50 c.c. of urine and add 60 c.c. of a 10 per cent. solution of neutral acetate of lead. The precipitate which takes place includes sulphuric and phosphoric acids, and part of the uric acid and chlorine, while the sugar remains in solution. Separate the precipitate on a filter, and treat the filtrate with an excess of ammonia. A further precipitate occurs, which contains the sugar in combination with plumbic oxide.

This precipitate is then collected and washed, great care being taken to remove all the ammonia, which is best effected by repeating several times the subsidence and decantation before throwing the precipitate on the filter; after which water is passed through until red litmus paper is no longer turned blue by the filtrate.*

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* Dr. Pavy cautions against washing with hot water. Although it expedites the process, there is danger of breaking up the combination between the plumbic oxide and the sugar, thereby losing some of the latter.

The precipitate, suspended in about 100 c.c. of water, is decomposed by passing through it a stream of sulphuretted hydrogen as long as a precipitate is produced. Filtration is then performed and the excess of sulphuretted hydrogen expelled by heat. The filtrate, a colorless fluid, is then evaporated over a water-bath to a volume equal to that of the original urine operated upon, that is, 50 c.c.

Dr. Pavy recommends the testing of the fluid thus obtained by the Fehling's solution, *but Prof. Wormley has discovered that it still contains uric acid which is capable of producing a neat reaction with the cupric test.* This uric acid he removes by simply allowing the fluid to stand twenty-four hours or longer, at the end of which time a considerable sediment of uric acid has fallen to the bottom of the vessel; and it is this acid which produces the reaction in urine in the hands of Brücke, Bence Jones, and Pavy, and ascribed by them to sugar. When the uric acid is wholly removed from normal urine, no reaction occurs; but if the slightest trace of sugar is present, it occurs, and the sugar is detected.

IX. Other Saccharine Substances.

Inosite.

Inosite or muscle sugar is sometimes found in the albuminuria of nephritis as well as in diabetes mellitus, and in the latter disease it has been found occasionally to substitute glucose, especially during convalescence; also in phthisis, the syphilitic cachexia, and in typhus fever. Gallois examined the urine of 102 patients for inosite and found it in seven only; five times in 30 cases of diabetes

along with sugar in variable quantity, and twice in 25 cases of albuminuria.

Recognition.—Inosite is obtained from urine as follows: After any albumin that may be present is removed, the urine is treated with neutral acetate of lead, until precipitation ceases. It is then filtered, and the warmed filtrate treated with basic acetate of lead so long as a precipitate occurs. It is better, however, to concentrate the urine to one-fourth its bulk over a water-bath before precipitation. The lead precipitate which contains the inosite combined with the lead oxide is collected after twelve hours, washed, suspended in water and decomposed with sulphuretted hydrogen. Out of the filtrate there is separated, after standing some time, a small quantity of uric acid. This is filtered out, the fluid concentrated as far as possible and treated while boiling with three to four times its volume of alcohol. If there arises a heavy precipitate adhering to the bottom of the glass, the hot alcoholic solution is simply poured off. But if a flocculent non-adhesive precipitate occurs, the hot solution is filtered through a hot funnel and allowed to cool. If, at the end of 24 hours, groups of inosite crystals appear, they are separated and washed with a little cool alcohol.

In this case it is advisable, in order that there may be no loss of inosite, to dissolve the precipitate obtained by the addition of hot alcohol in as small a quantity as possible of hot water, and precipitate a second time with three or four times its volume of hot water. If no crystals separate, ether is added to the clear, cold alcoholic filtrate, until, on shaking thoroughly, a milky cloudiness appears, and then the fluid is permitted to stand in the cold 24 hours. If not too small amount of ether is added (an excess does no harm), all the inosite present is precipitated in pearl-like shiny plates.

Inosite differs from cane-sugar in not undergoing vinous fermentation when treated with yeast, although its solutions readily take on the lactic fermentation when brought in contact with putrefying cheese. It does not reduce cupric tartate in solution with potassic hydrate, but changes it to an olive-green, and after a while a flocculent precipitate falls, and the supernatant fluid becomes blue, but on again

heating the solution the olive-green color is redeveloped. This reaction is sometimes observed in treating urine with Fehling's solution, as was originally pointed out by Dr. Ralfe, and it has been suggested by Dr. Oliver that it may be due to inosite. Should this be true, inosite would appear to be less rare than has been heretofore supposed, but it is doubtful whether a reaction so indistinctive should be allowed much stress as compared with the exact chemical process above described.

Fruit-sugar, or Lævulose.

Fruit-sugar sometimes occurs in urine only accompanying diabetic sugar. It is characterized by being non-crystallizable, and turning the plane of polarized light to the left instead of to the right. The specific rotary power diminishes as the temperature rises, while that of grape-sugar is independent. It is −108.3 at 0° C.; −99.44 at 14°; −97.1 at 17.5°; −52.5 at 87.2°, according to Tuchschmied. It reduces the salts of copper as does glucose, but in a less degree.

Recognition.—Since lævulose is always associated with glucose when it occurs in urine, and both reduce the salts of copper, it is only by their opposite action on polarized light that they can be distinguished. If, therefore, a sugar-holding urine deflects polarized light strongly to the left, or if, after allowing for the fact that normal urine turns it to the left 10, and that this property is increased by the ingestion of large doses of benzol, phenol, bromo- and nitro-benzol, chloral, and camphor, polarized light is not deflected at all, we may conclude that lævulose is present. It is to be remembered, that these substances also reduce copper salts. Other substances turning polarized light to the left must likewise be excluded.

Sugar of Milk, or Lactose. $C_{12}H_{22}O_{11} + H_2O$.

Lactose is sometimes found in small quantity in the urine of nursing women and females of animals. Dr. Ralfe refers to a case under his observation in the London Hospital, of a young married woman, aged 29, who was suckling an infant and suffering with debility and frequent micturition, whose urine contained as much as 3 per cent. of sugar. This occurred in three successive confinements, there being no sugar during pregnancy.

Lactose is characterized by crystallizing in white, or colorless, four-sided prisms with acuminated ends bounded by four triangles, by its turning polarized light to the right with a rotating power of + 59.3°, varying also with the degree of concentration; while that of grape-sugar is +53.1; by the fact that it reduces the salts of copper as does grape-sugar, and that it does not undergo the alcoholic fermentation with yeast. The fungi which convert milk-sugar into alcohol are cleft fungi. On the other hand, the lactic acid and butyric acid fermentation are readily entered upon.

Recognition.—A very strong reducing power and an otherwise inexplicable deflection to the right suggest milk-sugar. Especially is this suspicion justified if the urine is that of a nursing woman. It can be recognized with certainty only by isolating it from the urine, for the details of which the student is referred to the works on physiological chemistry by Gorup-Besanez or Hoppe-Seyler, or the elaborate treatises on urine analysis by Neubauer and Vogel or Salkowski.

X. Acetone and Acetone-producing Substances; Diacetone.

Often closely, though not necessarily, associated with glycosuria, are those conditions of the urine which respond to the tests for acetone.

Acetone.

When small quantities of acetone are present in urine, it can only be recognized in the distillate of the urine, but if considerable in amount its presence may be shown by both Legal's and Lieben's tests directly applied to the urine, but the former is to be preferred when urine itself is used.

Legal's Test.—A few crystals of sodium nitroprusside are dissolved in a little water in a test-tube to obtain a fresh solution. Add a few drops of this to three or four cubic centimetres of the urine to be tested and to the mixture enough liquor sodæ to secure a distinct alkaline reaction. A carmine red color ensues, whether the urine contains acetone or not. This, however, rapidly fades into a yellowish brown, but if 2 or 3 drops of concentrated acetic acid be now added, the red returns, after some seconds, and if much acetone is present, an intense deep purple color, following the course of the drops of acid. This, however, again quickly disappears.

Lieben's Iodoform Test.—To a portion of the distillate or urine add a small quantity of liquor potassæ, then a few drops of a solution of iodine and iodide of potassium.* If acetone is present a yellow precipitate of iodoform is produced *at once*. Should alcohol happen to be present in the distillate, the reaction takes place also, but more slowly, but with acetone it is immediate.

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*Friedlander's formula for this solution is, iodine 1.0; potas. iodide 2; distilled water 50.

The latter source of error may be altogether avoided by the modification suggested by Gunning, who uses ammonium hydrate and tincture of iodine. With these, alcohol causes no precipitate, while acetone does produce one of iodoform in addition to iodine, which falls as a black sediment, even though there be no acetone. The precipitated iodine is soon redissolved if the urine contains much acetone; if it contains but little, the iodoform crystals may be seen in 24 or 48 hours, resting in a thin layer upon the black precipitate of iodine.

Where considerable acetone is present, Lieben's test may be used, as suggested by Dr. Ralph, as follows:

About a drachm of liquor potassæ, containing 20 grains of iodide of potassium, is placed in a test-tube and boiled. A drachm of the suspected urine is then carefully floated on the surface. When the latter comes in contact with the hot alkaline solution, a ring of phosphates is formed, and, after a few minutes, if acetone or its allies are present, the ring will become yellow and studded with yellow points of iodoform. These in turn will sink through the ring of phosphates and be deposited in the bottom of the tube.

This mode of applying the test is subject to error, due to the fact that lactic acid and ethyl alcohol, both of which are found in urine, act similarly.

Hence, too, it is safest to work with the distillate.

The Indigo Reaction of Baeyer and Drewsen.—Heat a few crystals of nitro-benzaldehyde until dissolved. Allow the solution to cool, when the aldehyde separates a. a white cloud. Then add the suspected fluid (preferably its distillate), and make the mixture distinctly alkaline with dilute caustic soda. If acetone is present, there appears, first a yellow, then a green color, followed by an indigo-blue, in the course of ten minutes. If only traces of ace-

tone are present, the yellow fluid is shaken with a few drops of chloroform, when a distinct blue coloration of the chloroform takes place.

By this method acetone can be easily detected in a dilution of 1 part to 2500, if the distillate is used. Even with undistilled urine, by the aid of chloroform, it can be detected if present in the proportion of 1 to 1000.

Pyroracemic acid, aldehyde, and acetophenon are the only other substances producing the indigo reaction, and these have not, as yet, been found in urine.

Diacetic Acid.

Diacetic acid is characterized by striking a Bordeaux-red color with a solution of the chloride of iron, and urine containing it in sufficient quantity responds similarly. Other substances, however, strike the same reaction, as the salts of formic and acetic acids; also carbolic and salicylic acids; decomposition-products of antipyrin, kairin, and thallin. Diacetic acid also responds to the various tests for acetone.

The chloride of iron test, as is practiced by v. Jaksch, is as follows: To urine, as fresh as possible,* are added a few drops of a solution of chloride of iron. If a precipitation of phosphates takes place, it is removed by filtration, and the filtrate again treated with the chloride of iron. In case a Bordeaux-red color is produced, a portion of the urine is boiled and treated in the same way. Another portion of the fresh urine is acidulated with sulphuric acid and shaken with ether. If the reaction in the boiled urine occurs but

* It is exceedingly important that the urine should be fresh, or decomposition prevented, because diacetic acid is so quickly converted into acetone.

slightly, or not at all, if, after 24–48 hours, the red color produced by testing the ethereal extract grows pale, and if, operating on the distillate, the urine is found rich in acetone, we have, then, to do, according to v. Jaksch, with diacetic acid.

Le Nobel says the red color produced by Fe_2Cl_3 may also be produced by formic acid. He has found formic acid in 3 out of 7 cases of diabetes.*

Clinical Significance of Acetone and Diacetic Acid.—We are indebted to v. Jaksch† for most of our knowledge of the difference in the clinical significance of these two substances, which were formerly thought to be of identical import.

Acetone is found in cases whose course is usually favorable, and may have little or no significance. Diaceturia, on the other hand, is a most dangerous complication.

Acetonuria seems to be the result of continued high temperature, or at least accompanies diseases attended with such temperature, and a diminution of temperature is followed by a fall in the quantity of acetone, while a redevelopment of high temperature is followed by an increase in acetone.

Acetonuria often accompanies diabetes, but is not necessarily associated with it or with glycosuria, and while the development of acetonuria in diabetes is sometimes accompanied by very unpleasant symptoms, as headache, loss of appetite, and deranged digestion, all of short dura-

* Am. Jour. Med. Sci., Jan., 1887.

† Acetonurie und Diaceturie, Berlin, 1885. For a very careful presentation of the results of v. Jaksch's observations on this subject, see a paper by Dr. J. P. C. Griffith, in the Philadelphia Medical News, for October 3, 1885.

tion, it is otherwise of little significance except as a possible precursor of the diaceturia which sometimes succeeds it.

Among other diseases with which acetone has been found associated are carcinoma, inanition, and cerebral psychoses accompanied by mental excitement.

There is also a condition, a sort of auto-intoxication, or "acetonæmia," *sui generis,* in which the acetone would seem to be solely responsible for a set of symptoms in which restlessness, excitement, and delirium are the most conspicuous, and which may either pass away entirely, or terminate in coma and death.

On the other hand, what is commonly known as diabetic coma, according to v. Jaksch, is the result, not of acetone in the blood, but of *diacetic acid,* although he admits that it is often preceded by a long-continued acetonuria. It is commonly observed in cases of far-advanced diabetes. There is added to the usual feeling of weakness and depression drowsiness which may deepen into coma or pass away, the diaceturia continuing. As a rule, there is no relation between the amount of sugar and diacetic acid eliminated, although a sudden diminution of glycosuria is sometimes followed by the appearance of a large amount of diacetic acid, coma and death.

Diaceturia also sometimes accompanies mental diseases with excitement, and has been noted in inanition and carcinoma, so that a *coma carcinomatosum* has been described, similar to that of diabetes. It is to be remembered, however, that symptoms of diabetic coma may occur without either acetonuria or diaceturia. V. Jaksch proposes to do away with the term "diabetic coma" and substitute "coma diaceticum" for all of those cases of coma, from whatever remote cause, accompanied by diaceturia.

Finally, there appears also to be an auto-intoxication or "diaceticæmia" from diacetic acid, manifested by vomiting, dyspnœa, and jactitation, which soon terminates in coma and death, unattended by any other discoverable grave disease. This condition, very grave, but rare in adults, is said by v. Jaksch, to be much more frequent in children and correspondingly less serious. In such cases the child feels weak, has a thickly coated tongue, often slight conjunctival catarrh, sometimes vomiting, usually constipation, and very little or no fever. In two or three days, all of these symptoms, together with the diaceturia, disappear. In other cases nervous symptoms are more marked. V. Jacksch believes that all of these, as well as a certain number of other convulsive attacks in children, are the result of auto-intoxication with diacetic acid.

It is evident that our knowledge of these substances, acetone and diacetic acid, as well as that of the symptoms developed by their presence, is not yet definite; but the statements just made may be considered as representing as definitely as possible our present information.

β Oxybutyric acid. — Hugounenq,* Lepine, Stadelmann and Minkowski are inclined to believe that this substance and not diacetic acid is the cause of diabetic coma. It is found in the blood of diabetic patients; is the homologous superior of lactic acid; and is formed from the diseased muscle, just as lactic acid is from the healthy muscle. Diacetic acid is only a further oxidation of it. Just by what steps it is formed within the body we are unable to discover. The method by which the changes may be brought about outside of the body is, however, very simple; the series then running as follows: glucose, alcohol, aldehyde, aldol, β oxybutyric acid, diacetic acid, acetone. Hugounenq advises hypodermic injections of alkali to neutralize the acid in the

* Amer. Jour. Med. Sci., Oct., 1887.

blood. Lepine also recommends an alkaline treatment, and says an exclusive meat diet is to be avoided as favoring the development of coma.

These observers say, too, the acid found in the urine in many cases of diabetes is not diacetic acid but oxybutyric acid.

XI. Coloring Matters.

The pathological significance of all the coloring matters has not as yet been determined. Many of them are, however, of such importance that their consideration commands interest next to that of the proteids and sugars.

I. *Normal Coloring Matters.*

Notwithstanding the very considerable study which has been given to this subject of late years, there is still much confusion in regard to the normal coloring matters. The two as to the existence of which, as separate proximate principles, there is most satisfactory evidence are **urobilin** and **urine-indican**, the latter being the uroxanthin of Heller.

Thudichum* makes a single coloring matter which he calls *urochrome*. To this matter, according to Thudichum, the urine owes the whole or greater part of its yellow color, while numerous other coloring matters, including the urrhodin of Heller, Scherer's urohæmatin, and the urohæmatin of Harley, he considers mixtures of the products of decomposition of this yellow pigment.

The urohæmatin of Scherer and that of Harley are probably identical, Scherer† admitting that urohæmatin contains iron, and approving of the use of the term by

* Thudichum, A Treatise on the Pathology of the Urine, 2d edition London, 1877.

† Harley, The Urine and its Derangements, Philadelphia, 1872, from London edition, 1871.

Harley for his coloring matter. The urophain of Heller is probably the same thing. It will at any rate here be so considered. Finally, all of these are probably modifications, due to different methods of treating urine, of the substance known as *urobilin*, now generally acknowledged to be the most important coloring matter of the urine. Upon the presence of indican (Heller's uroxanthin) in most normal urines, all are agreed, although Thudichum prefers not to consider it a coloring matter, but a *chromogen* or color generator. For the present I shall retain it among the normal coloring matters, treating therefore chiefly of two, viz:

1. **Urobilin** (Jaffé), *hydrobilirubin* of Maly, and its modifications; *urohæmatin* of Harley and Scherer; *urophain* of Heller.

2. **Urine-indican** or the uroxanthin of Heller; to which will be added a short account of urochrome as described by Thudichum.

1. *Urobilin—Hydrobilirubin—Urophain—Urohæmatin.*

Urobilin, first extracted by Jaffé, and further studied by Maly, is believed to be the bilirubin or normal coloring matter of the bile—altered after passing into the small intestine, by absorbing water and hydrogen—in a word, reduced bilirubin. Hence Maly names it hydrobilirubin. The reaction may be expressed as follows:

$$\underset{\text{Bilirubin.}}{2(C_{16}H_{18}N_2O_3)} + H_2O + H_6 = \underset{\text{Urobilin}}{C_{32}H_{44}N_4O_7}.$$

As such it is reabsorbed and excreted by the kidneys. As bilirubin is itself the hæmatin of the blood, reduced by the action of the bile acids, the direct descent of urobilin from the blood is established. Obtained most readily

from high-colored fever urines by processes described in the larger works on urinalysis, it is a brown resinous mass, easily soluble in water, but more readily in alcohol, ether, and chloroform. It gives no play of colors with nitric acid. Its concentrated solutions are brown ; more diluted they are yellow, and still more, rose-red. When concentrated they exhibit peculiar spectroscopic properties and a beautiful green fluorescence by reflected light. The spectrum is a dark absorption band between Fraunhofer's lines b and F. Both properties become more distinct by the addition of solution of ammonia and a drop of chloride of calcium, while the fluorescence ceases on adding hydrochloric acid, and the absorption band recedes towards F, and becomes more indistinct.

Jaffé has inferred from the absence of these peculiar reactions of urobilin in fresh urine that urobilin is not at first present, but is preceded by a chromogen, which is converted into urobilin on exposure, by absorbing oxygen.

Test for Urobilin or Hydrobilirubin.—Add ammonia until distinctly alkaline, filter, and to the filtrate add a little chloride of zinc solution. The appearance of a green fluorescence and the characteristic absorption band indicates the presence of a considerable amount of bilirubin.

It is especially abundant in the high-colored urine of fever cases, heart and liver diseases, and after sweating.

Heller's test for urophain is as follows: About 2 c.c. (32.4 minims) of colorless sulphuric acid are poured into a small beaker-glass, or, better, a "collamore" wineglass (p. 17), and upon it in a fine stream, from a height of about four inches, twice as much urine is allowed to fall. The urine mingles intimately with the sulphuric acid, and in normal urine, of which the specific gravity is 1020 and

the quantity 1500 c.c. in the twenty-four hours, produces a deep garnet-red coloration.

If the coloring matter is increased, the coloration is no longer garnet-red, but is *black* and *opaque;* whereas, if the coloring matter is diminished, the mixture appears *pale garnet-red* and transparent.

Precautions.—Unfortunately, other conditions than that of increased amount of coloring matter produce the increased intensity of the urophain reaction. Thus diabetic urine produces the same dark opacity through carbonization of the sugar by the sulphuric acid. In like manner, urine containing blood, biliary coloring matters, and uroerythrin (an abnormal coloring matter), gives the same reaction with sulphuric acid. Before relying, therefore, upon this reaction, the above substances must be carefully excluded.

Dr. Harley's test for urohæmatin is as follows: Dilute the twenty-four hours' urine with water till it measures 60 ounces (1800 c.c.), or if the quantity exceeds 60 ounces, concentrate it to this amount; then to about 2 drachms (7.4 c.c.) of it, in a test-tube, add half a drachm (1.8 c.c.) of pure nitric acid, and allow the mixture to stand for some minutes. If the quantity of urohæmatin is normal, the mixture will alter but slightly in tint; whereas, if there be an excess, it will become pink, red, crimson, or purple, according to the amount present. Heating the mixture hastens the change in color, but it is better to do this experiment in the cold, and, if necessary, allow plenty of time for the change to take place.

The acid is added to liberate the coloring matter, which may be so thoroughly concealed that a *pale urine often contains a large amount of urohæmatin.*

Harley gives a second method, also easy of application, for determining an excess of urohæmatin in cases of

destructive diseases of the blood. Boil 4 ounces (120 c.c.) of urine, and add nitric acid to set the coloring matter free. When cool, put the urine in a six-ounce bottle along with an ounce of ether. Cork the bottle, thoroughly shake it, and place aside for twenty-four hours. At the end of that time the ether will be found to be like a red, tremulous jelly. Such a case, however, he admits to be a bad one.

There is every reason to believe that urohæmatin represents the disintegration of red blood corpuscles, and that it fluctuates, therefore, with the rate of their destruction.

Urochrome of Thudichum.—Thudichum terms the substance, to which he considers the whole or greater part of the yellow color of the urine is due, urochrome. It is an alkaloid, but not of pronounced basic properties. It has been isolated, but not finally analyzed. Its principal characteristic is that on chemolysis with acids it is split up into several bodies of smaller atomic weight, one of which —uromelanin—seems to be derived from the coloring ingredient of the blood. Urochrome does not show any specific absorption band before the spectroscope when strongly acidified, but by chemolysis probably gives rise to two or three substances having distinct spectrum phenomena which greatly aid in their diagnosis. It is not the chromogen of urobilin.

Thudichum gives (*op. cit.*) several methods of isolating urochrome, the briefest of which consists in precipitating fresh urine with neutral and basic lead acetate, decomposing the precipitate with sulphuric acid, and precipitating the urochrome and some xanthin-like body from the filtrate by phosphomolybdic acid.

2. *Urine-indican— Uroxanthin of Heller—Indigogen of Thudichum.*

Indican, $C_{52}H_{62}N_2O_{34}$, or **uroxanthin,** is itself a colorless substance, separable from urine in the shape of a clear brown syrup, easily soluble in water, alcohol, and ether. It has a bitter taste, and is easily converted by treatment with acids under warmth into *indigo-blue* (the uroglaucin of Heller), and a red coloring matter (urrhodin of Heller), said by Kletzinsky to be identical with indigo red; but this is denied by Thudichum. This is said to occur also as the result of putrefaction. Formerly one of these products was said to be indigo-*glucin,* a saccharine substance which is said to respond to Trommer's test, but not to the fermentation test. This is now denied. According to Thudichum, *urrhodin* is the result of chemolysis by acids of a separate chromogen which he calls *urrhodinogen.* Uroxanthin or urine-indican was formerly thought to be identical with plant-indican, but more recent investigations tend to show a difference.

Heller's test is performed as follows: 4 c.c. or f℥j of pure hydrochloric acid are poured into a smooth wine- or a small beaker-glass, and into the same while stirring 10 to 20 drops of urine are dropped. Under normal conditions indican is present in urine in so small quantity that the acid to which the urine is added is colored *pale yellowish-red.* If indican is present in larger amount, the coloration is *violet* or *blue.* The more abundant the indican the more rapid does the violet or blue coloration take place, and often 1 to 2 drops of urine are sufficient to color f℥j, or 4 c.c. hydrochloric acid. The blue color does not always make its appearance immediately. It is well then

to wait 10 or 15 minutes, but the reaction which appears after such an interval indicates but a small quantity of indican. The addition of 2 or 3 drops of pure nitric acid makes the test more delicate, and small amounts of indican are thus recognized. If it is desired to test urine containing the biliary coloring matters for indican, the former must be precipitated by solution of lead acetate and filtered out.

Jaffé's method is more striking in its results, and is even approximately quantitative. To 10 or 15 c.c. (2.7 or 3.24 f℥) of urine in a large test-tube add an equal amount of fuming hydrochloric acid, and then, with constant shaking, a *perfectly fresh* saturated solution of calcic hypochlorite (chloride of lime) drop by drop, until the greatest intensity of the blue color is reached. This is then shaken with chloroform, which readily dissolves the freshly formed indigo, and separates from the aqueous solution as a blue fluid, the color being more or less deep according to the amount of indican present. In pale urines, often very rich in indican, this method will serve to determine its amount with sufficient accuracy for clinical purposes. Dark urines, whose other coloring matters are also decomposed by hydrochloric acid and calcic hypochlorite, should first be decolorized by a solution of basic acetate of lead, avoiding a great excess of the latter, when, if indican is present, a good indigo extract can be obtained in this way.

Precaution.—Albumin must always be separated before performing this analysis, as well as Heller's test, as it develops a blue color with hydrochloric acid after standing a long time. If the resulting color is red instead of blue, iodine is present, and thus the absorption of iodides ascertained.

Clinical Significance of Indican in the Urine.—Normal urine, according to Jaffé, contains 4.5 to 19.5 milligrams in 1500 c.c., or about 6.6 in 1000 c.c. It is increased by a meat diet, in obstructive diseases of the bowel, in pyelitis, diseases of the spinal cord and its membranes, and especially derangements of the entire central and peripheral nervous system, in urina spastica, after coitus and in hot weather, probably from concentration of the urine. It is also especially abundant in the urine secreted during the reaction from cholera (Wyss).

It has been found by Neftel in cases of cancer of the liver; and its presence in large quantities in persons affected with malignant tumors, he considered pathognomonic of cancer of the liver; by Hoppe-Seyler, in a case of melanotic cancer of the orbit. Jaffé finds indican increased in all diseases attended by intestinal obstruction, cancer of the stomach, lymphoma and lympho-sarcoma in the abdomen, purulent peritonitis, certain forms of diarrhœa, and in various diseases where the latter is a symptom. Rosenstein found indican increased eleven to twelve times in Addison's disease.

I found it markedly increased in two cases of cirrhosis of the liver confirmed by post-mortem examination, and in one of evident malignant disease of some abdominal organ, probably the liver; but the diagnosis was not certain and there was no autopsy. M. Robin has recently announced that he considers the presence of indican a valuable diagnostic sign in typhoid fever.*

From these facts it is evident that it is difficult to associate it pathognomonically with any disease. But recent

* Philadelphia Medical Times, October 22, 1881, p. 63.

physiological observations afford a rational explanation for its increase, which is strikingly confirmed by the clinical observations above noted. Indican is increased when a substance known as *indol* (C_8H_9N), first discovered by Baeyer, is introduced into the blood. It was found by Kühne (Virchow's *Archiv*, vol. xxxix.) that during the artificial fermentation of albumin in the presence of minced pancreas, indol was produced. Jaffé suggested that the indol thus produced during digestion is absorbed and converted in the blood into urine-indican. Now it is supposed that in ordinary normal intestinal digestion very little indol is produced; but wherever digestion is interfered with or delayed, as is evidently likely to be the case in almost all of the conditions above instanced, more is produced, absorbed, oxidized, and excreted as indican, thus accounting for its presence in increased amount under the circumstances.

Uroglaucine.—Apery* has recently announced that he has found *uroglaucine* in every one of twelve cases of scarlet fever, deposited in small, blue masses so distinctive that they can scarcely be confounded with any other substance. Both uroglaucine and urrhodine are sometimes found in the sediment of cystitis and Bright's disease.

To obtain Uroglaucine.—Filter the urine and sediments. Dry the filtrate and treat with boiling alcohol, which dissolves out the blue materials, and on evaporation, the uroglaucine is left with certain other matters which are washed off with cold water. The uroglaucine is again treated with boiling alcohol and the blue crystals are obtained by careful evaporation.

Dr. Harley believes that all the various colored urine-pigments are but different grades of oxidation of urohæma-

* Apery, Les Nouveaux Remèdes, Nov. 1, 1885.

tin,* and thus accounts for the various cases of blue, green, brown, and black urines which have been at different times reported, a most important fact with regard to which is that they never exhibit these colors at the moment the urine is passed, but acquire them after exposure to the air or the action of chemical reagents. He considers these changes which occur in urohæmatin out of the body are primarily due to its constitution in the body having been altered by disease.

He admits, however, in common with others, that some portion of the coloring matter of the urine comes from the food, chiefly vegetable food.†

My friend Dr. S. Weir Mitchell has called attention to a peculiar greenish or yellowish-green coloration exhibited by the urine of those who are upon a diet of skimmed milk alone. This coloration is probably such as would be expected when the coloring matter derived from the hæmoglobin of the red blood-corpuscles is uninfluenced by coloring matters contained in food, but it is a subject which requires to be investigated.

II. *Abnormal Coloring Matters.*

Under abnormal coloring matters are included those which never enter into the composition of normal urine, whether found elsewhere in the body or not.

They include (a) *the coloring matters of blood*, hæmoglobin or oxyhæmoglobin, methæmoglobin, and hæmatin. Hæmatin is a deoxygenated hæmoglobin, into which and a coagulated albuminous substance the latter is converted by the action of heat. Methæmoglobin is an intermediate condition, approaching, however, nearer to hæmatin, and

* *Op. citat.*, p. 110.

† *Op. citat.*, p. 101, *ad fin.*

gives the same absorption band, in the yellow of the spectrum between Fraunhofer's lines C and D, but nearer to D, while hæmoglobin gives one band in the yellow and one in the green between D and E.

In fresh urine containing blood-coloring matters the prevailing one is hæmoglobin; but if a specimen of such urine be treated by sulphuret of ammonium, it becomes reduced hæmoglobin by loss of its oxygen. Of this, the spectrum gives a single broad band between the lines D and E. Shaking with oxygen or atmospheric air again restores the reduced hæmoglobin to oxyhæmoglobin.

(b) The *uroerythrin* of Heller.

(c) *Vegetable coloring matters.*

(d) *Biliary coloring matters.*

(a) *The Coloring Matters of the Blood—Hæmoglobin, Methæmoglobin and Hæmatin.*

These substances can enter the urine either by direct transudation, or arise from the dissolution of blood-corpuscles themselves, which have entered the urine in different ways. They may be present in the urine in very small quantities without being accompanied by albumin, as was first shown by Dr. F. A. Mahomed.*

The color of the urine is different according as it contains more hæmoglobin or methæmoglobin, the former being brighter, the latter darker, brownish-red. Hæmorrhages from the larger vessels produce more hæmoglobin; capillary hæmorrhages, on the other hand, more methæmoglobin. Heller proposes to account for the difference by

* Transactions of the Royal Medico-Chirurgical Society of London, vol. lvii., 1874, p. 196.

the fact that in the hæmorrhages which take place from the capillaries in renal disease, the blood is much more slowly and more intimately commingled with the urine, and therefore longer retained with it at the normal temperature of the body. Temperature, the presence of carbonic acid, and the absence of oxygen, may favor the change of hæmoglobin to methæmoglobin.

Detection of Blood-Coloring Matters.

1. **Mahomed's Test** *for Small Quantities of Hæmoglobin Unaccompanied by Albumin.*—Dr. Mahomed (*op. cit.*) directs as follows: One end of a small slip of white blotting-paper is dipped in the urine and dried over the flame of a spirit-lamp; by this means the dilute solution of the crystalloid is concentrated by evaporation; two drops of the tincture of guaiacum are then dropped on the paper, and, after a minute or so, allowed for the spirit to evaporate, a single drop of ozonic ether* is let fall in the centre of the guaiacum stain. A *blue* color appears if hæmoglobin is present. Some time, perhaps a quarter of an hour, will elapse before the reaction becomes visible, especially if it be slight; when it appears it is not permanent; it will begin to fade in a few hours, and will have disappeared in a day or two.

The advantage of this test lies in the fact that the physician can carry a few slips of blotting-paper in his pocket-book, dip one in the urine during his visit, allow it to dry, and make the test at home.

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* Ozonic ether may be obtained in Philadelphia, of L. Wolf, apothecary, northwest corner Twelfth and Chestnut streets. Both it and the tincture of guaiacum should be freshly prepared.

Dr. Stevenson's modification of Dr. Mahomed's test, acknowledged by the latter to be far more brilliant, is as follows: To a drop or two of urine in a small test-tube add one drop of the tincture of guaiacum and a few drops of ozonized ether; agitate and allow the ether to collect at the top, forming an upper layer of fluid. If hæmoglobin be present the ether carries up with it the blue color that is produced, leaving the urine colorless below. In this method the blotting-paper, which is somehow the source of fallacy, is not required.

Precautions.—Saliva, nasal mucus, and a salt of iodine (as happens when the patient is taking iodide of potassium) all strike a blue color with tincture of guaiacum, some without and some after the addition of ozonic ether.

Clinical Applications.—By this test, according to Dr. Mahomed, infinitesimal traces of hæmoglobin can be detected in urine which to the naked eye, the microscope, the spectroscope, and even to the nitric acid test for albumin, affords no indication whatever of abnormality. Indeed, the presence of albumin in any quantity interferes with the test, and it is in the *prealbuminuric* stages of scarlatina, or just after it has disappeared, and where there is a high state of vascular tension, that it is serviceable. It will respond in chronic albuminuria also, where minute traces of blood are present. Where the response precedes the appearance of albuminuria, it fades when the albumin becomes copious, and reappears again as it diminishes or after it disappears.

The most useful application of the test, if Dr. Mahomed's views are sustained, will be in the prealbuminuric stage of scarlatina, where it will give us information of a state of affairs in the kidney previous to actual inflammation of the

organ, when a brisk purge or copious sweat may avert more serious mischief. In cases of albuminuria produced by intense fever and due to venous congestion, as in enteric fever, pneumonia, and sometimes in the febrile stage of scarlatina, when the fever is intense and the albuminuria only slight, no reaction showing the transudation of the hæmoglobin can be obtained.

2. The presence of **hæmoglobinuria** as distinguished from **hæmaturia** is determined by the absence of blood disks and the presence of a smaller quantity of albumin, derived from the decomposition of the hæmoglobin. When a solution of hæmoglobin is heated in a test-tube it breaks up into a coagulated albuminous substance, hæmatin and methæmoglobin. The former is precipitated, not in flakes which quickly coalesce and form a large white bulky precipitate, as does coagulated serum-albumin, but forms a small, brownish, coherent coagulum which floats upon the surface. The color may be removed from the washed coagulum by boiling with alcohol containing sulphuric acid, the fluid becoming tinted reddish to reddish-brown, and given the spectrum of hæmaturia. Again, the color of such urine, although dark red in bulk, is yellowish and more transparent in thin layers than urine containing blood-corpuscles. It is of lower specific gravity than such blood, and deposits a less copious sediment.

It must not be concluded, that, because blood-corpuscles are absent from a given specimen of urine containing hæmoglobin, they have never been present; for they are sometimes rapidly dissolved, especially in alkaline urine. In such event we must depend upon the smaller amount of albumin just alluded to as characteristic of simple hæmoglobinuria, and the smaller sediment. The urine containing the dis-

solved corpuscles is more apt to be alkaline while the urine of hæmoglobin is acid in reaction. Should there be a transudation of serum at the same time with the hæmoglobin it would of course be impossible to distinguish the two.

3. **Heller's test for hæmatin** is as follows: Precipitate from urine in a test-tube the earthy phosphates by caustic potash and gentle heat, over a flame. The earthy phosphates carry with them, as they sink, the blood-coloring matters, and appear, therefore, not white as in normal urine, but *blood-red.* When the quantity of coloring matter in urine is very small, the earthy phosphates appear dichroic. If the urine is already alkaline, and no precipitate of earthy phosphate appears on the addition of liquor potassæ and heat, a precipitate can be artifically produced by the addition of one or two drops of the magnesian fluid, which, with the application of heat, carries down the coloring matters; whence it is possible.

To Prepare Hæmin Crystals.—If the precipitated earthy phosphates are filtered out and placed on an object-glass, and carefully warmed until the phosphates are completely dry, Teichmann's hæmin crystals can be produced therefrom. For this purpose a minute granule of common salt is carried on the point of a knife to the dried hæmatin and earthy phosphate, and thoroughly mixed with it. Any excess of salt is then removed, the mixture is covered with a thin glass cover, a hair interposed, and a drop or two of glacial acetic acid allowed to pass under. The slide is then carefully warmed until bubbles begin to make their appearance. After cooling, hæmin crystals can be seen by aid of the microscope. These, though often very small and incompletely crystallized, are easily recognizable by an amplification of 300 diameters. They are chemically hydrochlorate of hæmatin.

Precautions.—Care must, however, be taken to apply only a gentle heat in precipitating the earthy phosphates with caustic potash solution, and to filter quickly, lest the hæmatin may be decomposed.

It sometimes happens, also, that vesicles develop under the thin glass cover, after the addition of acetic acid, even before heat has been applied. These are carbonic acid. They should be allowed to pass away, and the slide then warmed until the formation of vesicles, that is, to the boiling-point of acetic acid.

Occurrence.—Hæmoglobinuria, that is the direct passage into the urine from the blood, of the coloring matters unaccompanied by the corpuscular element, occurs in certain general diseases, as scurvy, purpura, scarlatina, profound malarial poisoning, etc. Hæmaturic or bloody urine results from the above and from a variety of other causes which require no special mention.

Melanin is sometimes found in the urine of persons having melanotic cancer or sarcoma. It is deposited from urine in the shape of granular particles. These are soluble in liquor potassæ, and their solution is decolorized by passing chlorine through it. Melanin differs from carbon in being soluble in potash, while carbon is not.

(b) *Uroerythrin.*

Heller ascribes the well-known *dark reddish-yellow* or "high" color of all fever urines to the presence of a substance which he calls uroerythrin, as well as to an increase of the normal coloring matters. Except that it contains iron, little else that is certain is known with regard to uroerythrin. To it he ascribes the reddish color which so often characterizes the deposits of urates known as "lateritious;" if the supernatant urine in such cases be treated with solution of neutral acetate of lead, the precipitate presents

a similar "rosy red" or "flesh color," which he attributes to the same substance. It is doubtless a modified hæmatin, being found especially in diseases where there is evident blood dyscrasia, as in low fevers, septic conditions, etc. It so far at least corresponds with the urohæmatin of Harley that it is a measure of the destruction of the blood-corpuscles, though it will be remembered that the urohæmatin of Harley is looked upon as a normal constituent of urine which may be abnormally increased, while uroerythrin, although a modified hæmatin, is still not considered identical by its discoverer.

Neubauer includes uroerythrin among the normal coloring matters, while Hoffmann and Ultzmann, following Heller, treat it as abnormal.

Detection.—Uroerythrin is known to be present by its pink coloration of the "lateritious" sediment, or by its precipitation by solution of neutral acetate of lead. Too much lead solution must not be added lest the precipitate be too abundant, and the coloring matter rendered less distinct by its being disseminated over a large amount of deposit. If the urine contains hæmatin or the coloring matter of blood, it must be first removed.

Precautions.—1. The froth of a urine highly charged with uroerythrin may appear yellow, as that of urine containing biliary coloring matter, but the precipitate of the latter by acetate of lead is also yellow and not pink, as is uroerythrin.

2. The earthy phosphates which are precipitated on heating the urine with caustic potash are "dirty-gray" when the urine contains uroerythrin, while in urine containing hæmatin they are "blood-red" or dichoric. The absence of albumin from the urine, the gray coloration of the earthy phosphates, and the red precipitate with solutions of lead, serve as points in the differential diagnosis between uroerythrin and the coloring matter of the blood.

Clinical Significance.—Uroerythrin is found in the urine in all febrile affections, however slight; also, it is said, in pyæmia, diseases of the liver, and lead colic. All urine, according to Heller, which contains uroerythrin must be abnormal.

(c) *Vegetable Coloring Matters.*

The coloring matter of plants, especially chrysophanic acid, found in rhubarb and senna leaves, contributes to alkaline urine a reddish-yellow to a deep-red color. It can be recognized by the fact that the red alkaline urine on adding an acid becomes yellow, and on the addition of an excess of ammonia again takes on the red color.

Precautions.—Such precipitation by heat and potash solution might possibly be taken for blood-coloring matters. But the absence of albumin from the urine, the production of the red color by addition of an excess of ammonia, and its paling on the further addition of an excess of acid, serve to distinguish this vegetable coloring matter from blood-coloring matter and uroerythrin.

Numerous other vegetable matters color the urine, among which *santonin* is conspicuous for the bright yellow color it produces in acid urine, while the staining of linen by it closely resembles that of biliary coloring matter. Dr. W. G. Smith (*Dublin Quarterly Journal of Medical Science*, November, 1870) has investigated the subject, and found that the addition of alkali causes the development of a *fine cherry-red or crimson color*, according to the amount of santonin present; but it will be observed that this reaction is that of the vegetable-coloring matters generally, as above described.

Madder, indigo, gamboge, rhubarb, logwood, carrots, whortleberries, etc., give to urine more or less of their peculiar color.

Salicylic acid when administered in sufficient doses gives a smoky hue to the urine, and the urine strikes a blue color when a few drops of a solution of ferric chloride are added.

Carbolic acid introduced into the system in sufficient quantity causes a dark and even black discoloration of urine.

(d) *Biliary Coloring Matters—The Detection of Bile in the Urine.*

The biliary coloring matters are chiefly **bilirubin** ($C_{16}H_{18}N_2O_3$), **biliverdin** ($C_{16}H_{20}N_2O_5$), and **bilifuscin** ($C_{16}H_{22}N_2O_6$), the last two being derivatives by oxidation of the former. The last is found as such in herbivorous bile, and bilifuscin can be obtained from human gall-stones. None of these give any spectrum unless acted upon by reagents. We have seen that urobilin, the normal coloring matter of urine, is bilirubin altered by taking up, while in the small intestine, water and hydrogen, as the result of which it acquires the spectrum described on page 115. From the intestine it is absorbed, and excreted by the kidneys as the normal coloring matter of urine.

When bile is abundantly present in urine, the yellow color of the fluid, and especially of the froth or foam produced by shaking, is sufficient to excite suspicion. Further, if a piece of filtering-paper or a piece of linen be moistened with such urine, it retains a permanent yellow color on drying.

The only positive proof of the presence of the coloring matters of bile in the urine is found in Gmelin's or Heller's test for the unaltered coloring matters.

Gmelin's nitrous acid test is performed in two ways:

First. A quantity of urine is placed in a test-tube, and a small quantity of fuming nitric acid (nitrous acid of commerce) is allowed to pass carefully down the sides of the test-tube to underlie the urine, as described in Heller's test for albumin. If biliary coloring matters are present, at the point of union between the urine and the acid will very soon be seen a set of colors which, if typical, should be *green*, *blue*, *violet red* and *yellow*, or yellowish-green again, in the order named from above downward. Often, however, one or more colors are wanting. The green is most constant, and the *first green indispensable* to prove the presence of bile; but violet, shading into red and yellow, is also very constantly seen. The other colors may be produced by other coloring matters, especially indican.

Second. Equally satisfactory is the test if a few drops of the urine are placed upon a porcelain plate, and as much of the fuming acid is placed adjacent and allowed gradually to approach the urine. The same play of colors occurs.

E. Fleischl's Test.*—A modification of Gmelin's test, by which it is made more delicate. Instead of having impure nitric acid added in such a way that it will form a separate layer at the bottom, the urine should be thoroughly mixed with pure nitric acid, or, still better, with a concentrated solution of the nitrate of sodium, and then concen-

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* Boston Med. and Surg. Journal, Jan. 13, 1876, from Centralblatt für die medicinischen Wissenschaften, 1875, No. 34.

trated sulphuric acid should be carefully added so as to form a separate layer at the bottom. The play of colors forms at the junction of the urine and the sulphuric acid, the green appearing first above the acid, but rising gradually and giving place to the blue, violet-blue, and yellow. The advantage of this modification is that the pigment is not oxidized so rapidly, and therefore the color is not changed so quickly, remaining often half an hour or longer.

Heller's Test for Bile Pigment.—Pour into a test-tube about 6 c.c. (1.6 f℥) of pure hydrochloric acid, and add to it, drop by drop, just sufficient urine to distinctly color it. The two are mixed and "underlaid" as before with pure nitric acid, and at the point of contact between the mixture and the colorless nitric acid a handsome play of colors appears. If the "underlaid" nitric acid is now stirred with a glass rod, the set of colors which were superimposed upon one another now appear alongside of each other in the entire mixture, and should be studied by transmitted light. Heller further says, if the hydrochloric acid on addition of the biliary urine is colored *reddish-yellow*, the coloring matter is *bilirubin;* on the other hand, if it is colored *green* it is biliverdin.

If the amount of coloring matter is very small, a large quantity of urine should be shaken with chloroform; the chloroform allowed to separate at the bottom of the vessel in large drops. The yellow-colored chloroform is then removed by means of a pipette, washed with distilled water, and poured into a beaker-glass containing hydrochloric acid. The yellow drops of chloroform sink to the bottom. If now, while diligently shaking the glass, nitric acid is added, the changes of color can be distinctly

observed in the chloroform. In consequence of the slower action of the acid upon the coloring matters dissolved in the urine and the consequent slower transition of colors, this method is peculiarly adapted for demonstration.

Precautions.—1. With neither test should too dark-hued a urine be employed. Very dark urines should first be diluted with water.

2. Should albumin be present, the opaque zone at the point of contact between the urine and acid imbibes the coloring matters and exhibits a green coloration; so that the test is in no way interfered with.

3. Urine *rich in indican* may, however, deceive, forming at the point of contact a blue layer of indigo, which, along with the yellow urine, in reflected light, may appear green. In these doubtful cases the chloroform modification of the test should be used, or the urine may be precipitated with solution of acetate of lead, and the filtrate examined for indican.

4. The earthy phosphates, precipitated from biliary urine by liquor potassæ and heat, exhibit a brown coloration.

Ultzmann's Test.—Add to 10 c.c. of urine 3 or 4 c.c. of pure caustic potash solution (1 part of KOH to 3 of H_2O), then shake and add an excess of pure hydrochloric acid. The mixture assumes a beautiful emerald-green color.

Marechalt's Test.—Upon a specimen of urine in a test-tube allow a few drops of tincture of iodine to fall carefully. If biliary pigments are present a green color appears at the point of contact between the two fluids, and remains for some time, even twenty-four hours. In this test the possibility of confounding indican is said to be excluded.

Test for Decomposed Biliary Coloring Matters.—Should the urine contain only altered biliary coloring matters, which respond to neither Gmelin's nor Heller's test, Hoffman and Ultzmann recommend the following:

A piece of white linen or filtering-paper is immersed in the suspected urine, and allowed to dry, when it will appear colored brown. A further confirmation that the decomposed coloring matters are present will be found in a low specific gravity and a dark urophain reaction.* If, moreover, the urine be treated with liquor potassæ and heat, to precipitate the earthy phosphates, it becomes darker than before and the phosphates are precipitated brown.

Bile-pigments have a property of adhering to precipitates much more tenaciously than other pigments, and therefore sometimes cannot be detected in fluid urine when they may be in precipitates. Hence Dr. J. F. Tarchanoff (*Centrablatt für die medicinischen Wissenschaften*, 1875, No. 6) recommends, in order to separate with certainty the biliary from the urinary pigments, precipitating the urine with milk of lime, freeing from excess of lime by a current of carbonic acid gas, allowing the whole to stand a few hours, filtering, and washing the precipitate with water. The bile-pigments are contained in the precipitate, while the indican, hæmoglobin, and methæmoglobin are in the filtrate. The precipitate is then dissolved in acetic acid and tested by Gmelin's test.

In addition to urobilin the normal coloring matter of urine, directly derived by oxidation from bilirubin the normal coloring matter of fresh human bile, the latter substance and other derivatives from it are found in the urine when from any cause bile is reabsorbed. No attempt has been here made to separate these from each other, and the tests given are those for bilirubin and its combined derivatives, whatever they may

* It should be remembered that this dark urophain reaction is also produced by sugar and blood-coloring matters. These causes should, therefore, be eliminated.

be. Indeed I do not know that any of these except urobilin has been recognized in urine as a distinct coloring matter, unless it be the one last discovered by Stokvis,*—and apparently not yet named,—as a secondary product in most cases of the oxidation of biliary coloring matter, whereby Gmelin's reaction is produced. This same substance MacMunn† believes he has found in the urine, by the aid of the spectroscope, in certain cases of rheumatic fever, pregnancy, thoracic aneurism, cirrhosis of the liver, and cancer of the pylorus. It is indicated by an absorption band, which occurs on the red side of the band of urobilin.

In testing for it, the liquid is to be precipitated with lead acetate, excess of lead removed by oxalic acid, and the filtrate concentrated and boiled with alkalies and a reducing agent. If no reduction takes place, and if the other tests for biliary coloring matters have given a negative result, their absence may be inferred.

XII. The Biliary Acids.

From a perusal of almost all of the text-books on physiology, and even of numerous manuals on the examination of urine, the student is led to suppose that the detection of bile acids, if present in urine, by means of what is called Pettenkofer's test, is one of the easiest possible. This is, however, far from being the case, and the fact is that *such detection by the direct application of the elements of Pettenkofer's test in urine, or any other animal fluid, is practically impossible, even if the bile acids are present in considerable amount.* Nor have any of the modifications of Pettenkofer's test, recently announced as clinically available, proved such in my hands, even where the elements of bile have been added to the urine, except where inspissated ox-bile has been used. The results of a complete investi-

* N. Rep. Pharm., xxi., 123; Watt's Dictionary, 2d Supp., 1875.
† Spectroscope in Medicine, London, 1880, p. 168.

gation of this subject in its practical bearings will be found in a clinical lecture by the writer, in the *Philadelphia Medical Times* for July 5, 1873, "On a Case of Jaundice, with Remarks on the Availability of Pettenkofer's Test," to which the reader is referred. In these experiments the simplest method of obtaining the biliary acids was found to be as follows: Six or eight ounces (180–240 c.c.) of the suspected urine are evaporated to dryness over a water-bath. The residue thus obtained is treated with an excess of absolute alcohol, filtered, and the filtrate treated with an excess of ether (12 to 24 times its bulk), by which the bile-acids, if present, are precipitated. These are then removed by filtration and redissolved in distilled water. The solution is then decolorized by passing through animal charcoal and the resulting colorless fluid tried by Pettenkofer's test as follows: A single drop of a 20 per cent. solution of cane-sugar (simple syrup of the Pharmacopœia is many times too strong) is then added to a drachm or two (3.7–7.4 c.c.) in a test-tube or porcelain capsule. Sulphuric acid is then added drop by drop, while the test-tube is kept in a vessel of cold water, to prevent too great a rise in temperature, which should not exceed 50°–70° C. (122°–158° F.). As the quantity added approaches a bulk equal to that of the fluid to be tested, a beautiful *cherry-red* or *purple-violet* color should make its appearance. So soon as a yellow color appears, then the sulphuric acid is acting on the sugar, and the cherry-red can no longer be looked for. This carbonizing of the sugar is obviated by keeping the temperature down to the degree mentioned.

Even this method involves more time than is often available to the active practitioner, but there is none more

simple, and there is really rarely any necessity for any other than the color test, for the presence of the biliary acids, although undoubtedly occurring, is very rare, and the circumstances under which they occur are illy determined. It is not true, as was once supposed, that they are always present in the urine in cases of jaundice from *obstruction* and consequent *reabsorption* of bile (hepatogenous jaundice), and absent in cases of jaundice from dissolution of the blood (hæmatogenous jaundice), else would the determination of their presence be of real value in diagnosis. The only circumstances under which they are undoubtedly present in the urine are *rapidly* destructive diseases of the liver, as acute yellow atrophy and phosphorus poisoning. On the other hand, traces of the bile acids are said to be present in normal urine, Dragendorf having found .7 to .8 gram in 100 litres.* The bile acids yield a spectrum, which MacMunn has investigated. It gives a band outside D, and a broad band at E.

Dr. Oliver's New Peptone Test for the Bile-Acids.—This is founded upon the physiological fact that when the products of gastric digestion, peptone and para-peptone, which leave the stomach in an acid solution, meet with the bile, they are thrown down in a tenacious layer over the entire mucous membrane of the duodenum. So, too, albuminous urine or urine charged with peptone is precipitated by a solution of bile salts or of their derivative, cholate of sodium. Hence acidified albuminous urine becomes a test for bile salts, but an acidulated antiseptic

* The presence of bile-acids in normal urine is also asserted by Dr. Oliver, this assertion being based on experience with his new peptone test considered in the ensuing section.

solution of peptone is a readier and more delicate reagent. Such a solution is made by Dr. Oliver as follows:

Pulverized peptone (Savory and Moore),	gr. xxx.
Salicylic acid,	gr. iv.
Acetic acid,	♏ xxx.
Distilled water,	to f℥viii.

Perfect transparency is secured by repeated filtration.

Application.—The urine should be perfectly clear, rendered so by filtration if necessary, boiled and filtered if bloody, rendered normally acid if alkaline, and finally reduced to a specific gravity of 1008. Twenty minims should then be run into 60 minims of the test solution, and if the proportion of bile salts is normal or subnormal there is no immediate reaction, but in a little while there is a mere tinge of milkiness. If, however, the bile salts are present in excess, a distinct milkiness promptly appears, becoming more intense in a minute or two, the degree of opacity being directly proportionate to the amount of bile derivatives.

On agitation, the opalescence diminishes and perhaps finally vanishes, but is restored on adding more of the test solution. The precipitate differs from all other urinary precipitates induced by an acidified reagent, in dissolving completely on adding a drop or two of acetic acid or a citric acid test-paper, and by diminishing but not disappearing when boiled, but the opacity is not affected by such a degree of warmth as is sufficient to dissolve urates. Further an insufficiency, as well as an excess of acid, interferes with the reaction, as also does an excess of proteids, or of the salts themselves. Hence the importance of securing the proper proportions as in Dr. Oliver's formula, and of diluting the urine to be operated upon to a specific gravity of 1008. By the dilution is also secured such a solution of the

urates as avoids their precipitation and also any error consequent thereon. The reduction in specific gravity also obviates another source of error, in that concentrated urines often simulate an excess, while urines of low specific gravity, though affording a reaction similar to normal urine, may actually contain more than the normal amount of bile salts.

The test may be used in the contact method, by running the solution over the urine reduced in specific gravity to 1008. If the bile salts are present in normal amount or less, there is again no immediate response, but in the course of a minute, a delicate thread-like line makes its appearance, which may increase slightly. If the bile salts are abnormally increased an immediate reaction takes place.

This test, according to Dr. Oliver, is so delicate that there can readily be detected one part of bile salts in at least 18,000 to 20,000 parts of a solution of chloride of sodium. So far, he has been unable to find any other constituent of urine which reacts similarly, and, although it is true that a concentrated solution of chloride of sodium in the presence of an acid will precipitate a proteid, experiment shows that when the peptone solution is run upon a solution of salt of any specific gravity below 1050, no precipitation takes place. Hence there can be no error from this source in urine.

Mucin may be eliminated as a source of error, because this substance in acid solution is not precipitated by adding more acid, and when it is thrown down in urine of acid reaction, it is highly probable that the acid is not the reagent producing it, but merely supplies the requisite degree of acidity to enable the precipitant already present

to operate, and in that event, the mucin would only indicate the presence of bile salts.

Quantitative Estimation.—This is based upon a permanent standard of opacity provided by mixing together, in equal proportions, the test solution and normal urine reduced to the specific gravity of 1008.

To 60 minims of the test solution add the suspected urine reduced to a specific gravity of 1008, usually 10 to 20 minims at a time, allowing a minute to elapse after each addition, until the opacity induced is exactly equal to or slightly exceeds that of the standard, the tubes being held to the light, shaded by a dark background, such as a coat-sleeve.

If 50 or 60 minims bring up the opacity to that of the standard, the proportion of bile salts does not exceed the normal amount. Any smaller quantity required indicates an excess, while the smaller the amount needed, the larger the proportion of bile salts present.

Dr. Oliver has constructed a table showing the percentage of increase indicated by a varying number of drops:

Minims.		Drops.		Percentage of Increase on the Normal Standard.
1	or	2	=	6,000
2	or	4	=	3,000
3	or	6	=	2,000
4	or	8	=	1,500
5	or	10	=	1,200
10	or	20	=	600
15	or	30	=	400
20	or	40	=	300
25	or	50	=	240
30	or	60	=	100
35	or	70	=	83
40	or	80	=	66
45	or	90	=	50

An increase beyond 700 per cent. over the normal is rarely met, although Dr. Oliver mentions an instance of non-jaundiced urine which showed an increase of over 1500 per cent., which afforded at once a beautiful reaction with Pettenkofer's test.

Peptone Test Paper.—Dr. Oliver has also constructed a peptone test-paper which he considers permanent and reliable, and best used as follows:

The peptone paper with half a citric paper is dropped into 60 minims water in a test-tube or wineglass. After the lapse of a minute the solution is slightly agitated, and on being set aside for another minute is ready for use.

The solution thus prepared is taken up by the pipette and carefully run over the transparent urine. If bile salts are present in larger amount than the normal average, an immediate reaction is observed as a pearly-white thread or band. In urine in which there is no such excess, a delicate zone may appear, but only in the course of one or two minutes.

XIII. Leucin ($C_6H_{13}NO_2$) and Tyrosin ($C_9H_{11}NO_3$).

Leucin and tyrosin, products of a retrograde metamorphosis of nitrogenous substances, are found physiologically only in certain fetid secretions, as those of the axilla and between the toes, but can be produced by chemical means from some glands, as the liver, pancreas, and spleen, where they also occur in certain pathological states. They are found in urine, chiefly in rapidly destructive diseases of the liver, as acute yellow atrophy or phosphorus poisoning, but occasionally also in typhus and small-pox. They always accompany a large amount of biliary coloring matter and the presence of albumin. When at all abundant, as

they generally are in acute yellow atrophy, they are deposited from urine and are found in the sediment, the former in the shape of centrically marked spheres, arranged in warty masses, or druses, the latter in needles. (Fig 27.)

Schultzen has shown* that in animals poisoned by phosphorus, "urea disappears from the urine, and is replaced by leucin and tyrosin, which in the healthy organism are converted into urea." A similar substitution takes place in cases of acute atrophy of the liver, the retained urea accounting for the convulsive attacks which usually precede death in these cases.

Detection.—If the crystals, to be more fully described in treating of sediments, do not present themselves in the spontaneous deposit of such cases, the evaporation of a small quantity of the urine will generally promptly display them.

If they are not sufficiently abundant to be thus demonstrated, the method of Frerichs must be pursued to separate them. A large amount of urine is precipitated with basic acetate of lead, filtered, the excess of lead removed from the filtrate by sulphuretted hydrogen, and the clear fluid evaporated to a small volume over a water-bath. In twenty-four hours tyrosin needles will be found to have crystallized out, but leucin spheres will not appear until later, because of the great solubility of the leucin.†

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* Boston Medical and Surgical Journal, July 23, 1874, from Zeitschrift für Biologie, viii., 124, and Berliner klin. Wochenschrift, 1872, p. 417.

† Leucin and tyrosin are more fully treated by the writer in the American Journal of the Medical Sciences for January, 1872. The above is believed to be sufficient for practical purposes.

XIV. Fatty Matters.

That a trace of fat exists dissolved in normal urine has been shown by Schunk; while the list of reported cases in which fat is present in abnormal quantity is gradually increasing. In such cases are, of course, not included those in which fatty epithelium, fatty casts, and free oil drops are present, as the result of chronic Bright's disease; nor those in which fatty epithelium from the bladder or vagina occurs.

The oil may be present in urine in a state of minute subdivision into small drops and molecules as in the so-called chylous urine, or in the form of clear fluid oil. In the former instance the admixture probably results from the leakage of a lymph vessel into some part of the urinary tract. In the latter its source has been, in one instance* at least, traced to an abscess in the left lumbar region communicating with the left ureter; and such possible source should always be remembered. It is not impossible also that it may come directly from the kidney in cases of cystic cheesy degeneration of that organ, instances of which I have seen where there has been considerable free oil with compound granule cells and cholesterin plates among the cheesy matter. Dr. Roberts† refers to three cases in which pure yellow oil was present in the urine, in two during the administration of cod-liver oil, and in the third during the use of an emulsion. Dr. Henderson‡ reported three cases of heart disease in which free oil globules were suspended through the urine.

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* Dr. E. W. Cushing, in Boston Medical and Surgical Journal, vol. 104, 1880, p. 242.

† Urinary and Renal Diseases, Am. ed., 1879, p. 125.

‡ British Medical Journal, May 22, 1858.

Fat has been found in the urine from cases of calculous disease of the pancreas, and in one case referred to by Dr. George W. Johnston,* fat made its appearance in the urine one month before it was detected in the alvine dejecta, and in such quantity as to float, when cool, in greasy flakes on the surface.

The presence of **cholesterin** in the urine is also a possible but very rare occurrence, and may be conceived to occur in such a case of cheesy cystic kidney above alluded to. The only well-authenticated case I have ever seen reported is that given by Dr. Roberts.† Dr. Beale has shown that cholesterin may be obtained by treating large quantities of urine from cases of chronic Bright's disease, but this is a different matter from its being contained in urine in the free state.

XV. Urea (CN_2H_4O).

Urea is the chief organic constituent of urine and the index of nitrogenous excretion. Its quantity fluctuates with changes in the quantity and composition of ingesta, and with the rapidity of tissue metamorphosis in health and disease. A range of at least from 20 to 40 grams (308.6 to 617.2 grains) must be admitted in adults.

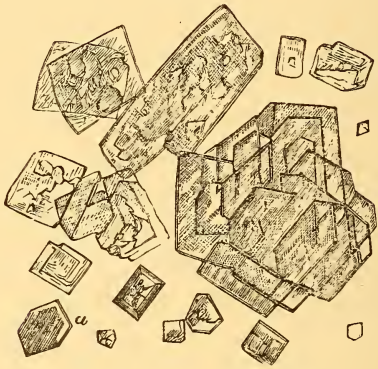
Detection and Estimation.—The odor of urine highly charged with urea may be said to be characteristic, but certain evidence of its presence can only be obtained by treating the solution suspected to contain it with nitric or oxalic acid. Though crystallizing itself in glistening

* Inaugural thesis for the degree of Doctor of Medicine in the University of Pennsylvania, 1882. Published in the American Jour. of the Med. Sciences, Oct., 1883, of which see p. 427.

† *Op. citat.*, p. 125.

needles, it is too soluble to permit of easy detection by its own form. If it be desired to detect its presence in a suspected fluid, a drop or two is placed upon a glass slide, a drop of nitric acid added, the slide carefully warmed over a spirit-lamp, and placed aside to crystallize. If urea is present, the microscope will reveal, singly or in strata, six-sided and quadrilateral plates of nitrate of urea (Fig. 13). The crystals have acute angles measuring about 82°, and

FIG. 13.



Crystals of nitrate of urea. (After Beale.)

are so characteristic as to be easily recognizable; they often overlap each other like the shingles of a roof.

Solution of oxalic acid produces similar but less regular crystals of oxalate of urea.

In ordinary normal urine, this crystallization does not take place unless the urine is concentrated by evaporation. But in some urines highly charged with urea, it is simply necessary to add nitric acid to produce the crystals, and thus is arrived at a rough quantitative estimation of urea.

As urea is by far the most abundant solid constituent of the urine, it follows that the specific gravity may become a means of approximately estimating its amount, especially when there is no sugar present, if the quantity of albumin is small and that of the chlorides is normal. A specimen of urine neither albuminous nor saccharine, containing a normal proportion of chlorides, and having a specific gravity of 1020–4 to a quantity of 1500 c.c. (50 oz.) in twenty-four hours, may be taken as a standard normal specimen containing 2 per cent. to 2½ per cent. of urea. These conditions being observed, a higher specific gravity would indicate an increased proportion, and a lower, a diminished proportion. Under these circumstances, a specific gravity of 1014 indicates about 1 per cent. of urea, and of 1028 to 1030 about 3 per cent.

But the chlorides fluctuate markedly in some diseases, and by far the largest proportion of urines in which a knowledge of the amount of urea is important contain albumin. Next to urea, supposing albumin and sugar absent, the chlorides most affect the specific gravity, being separated to the amount of 10 to 16 grams (154 to 247 grains), or ⅔ to 1 per cent. in the twenty-four hours. If these are totally absent, as they often are in pneumonia and other febrile diseases, accompanied by an increase in the elimination of urea, then must a specific gravity of 1020 indicate more than 2½ per cent. of urea, or, if the percentage of chlorides replaced by urea be added, 3½ per cent. This is on the supposition, of course, that the remaining constituents, uric acid, creatinin, phosphates, sulphates, etc., have little influence on the specific gravity —which is the fact.

If albumin is present in small quantity, not exceeding $\frac{2}{10}$

per cent., it has little effect, and it can be thrown out of the question. If, however, the albumin be more abundant, 1 to 2 per cent., it must first be removed by coagulation and filtration, and the approximate estimation be made from the specific gravity of the filtrate after cooling. Care must of course be taken to wash the coagulum by further addition of water until the quantity of fluid originally operated with is restored. After such removal of albumin, if not before, the specific gravity will generally be found lower than in health, showing—what volumetric analysis has determined more precisely—that in chronic albuminuria, at least, the quantity of urea is generally diminished.

Where sugar is present, the *percentage* of urea is also generally less, though with increased specific gravity, while the large total quantity of urine in the twenty-four hours may show an increase in the total urea for the day. There is no way of allowing here for the specific gravity due to the presence of sugar, and the only way to arrive at a knowledge of the amount of urea is by volumetric analysis.

Volumetric Analysis for Urea.

Under any circumstances, when an accurate estimation of urea is required, we must have recourse to volumetric analysis. Several methods of volumetric analysis for urea have been suggested, of which that of Liebig, with the nitrate of mercury solution, seems most to combine accuracy and convenience. Davy's method, with the sodium hypochlorite and pure mercury, is, in some respects, more simple, but it is also more liable to error, and really takes more time for its completion, while Liebig's process is carried out with surprising celerity, after even a little experi-

ence, not more than fifteen minutes being required to complete it if the solutions are at hand. Liebig's process is based upon the fact that urea produces a precipitate with mercuric nitrate.

The following test-solutions are required:

1. *The baryta solution,* consisting of one volume of cold saturated solution of barium nitrate with two volumes of cold saturated solution of caustic baryta (barium hydrate).

2. A saturated solution of sodium carbonate.

3. *A standard solution of mercuric nitrate* of such strength that 1 c.c. is precisely equivalent to .010 gram, or 10 milligrams, of urea.

To Prepare the Standard Solution of Mercuric Nitrate.—1. Dissolve 71.48 grams of pure mercury or 77.2 grams of the mercuric oxide in nitric acid by the aid of heat. The acid fluid is concentrated by evaporating over a water-bath to a syrupy consistence, and then diluted with distilled water to a volume somewhat less than a liter. If on dilution a white precipitate of basic nitrate of mercury fall, allow it to settle, and decant the clear liquid. Then add to the residue a few drops of nitric acid to dissolve the precipitate. Add the solution thus obtained to the former decanted liquid, and dilute to exactly one liter. The solution requires to be graduated by

2. **The Standard Solution of Urea.**—Two grams of pure urea should now be dissolved in 100 c.c. of distilled water, of which 10 c.c. will then contain 0.2 gram or 200 milligrams.

Ten c.c. of the standard solution, containing 200 milligrams of urea, are now placed in a beaker-glass. A burette is then filled to 0 with the solution of mercuric nitrate (taking care that the lower edge of the meniscus which forms the upper surface of the liquid corresponds with the arrow on the burette), which is then allowed to drop into the beaker, where it will quickly form a dense precipitate. When the precipitation seems about complete, a drop of the fluid containing it is allowed to fall on a drop of the solution of sodium carbonate, of which several are previously

ready on a piece of glass on a dark ground. If the urea is not completely precipitated, no change of color takes place. The cautious addition of the mercuric nitrate is continued, also the process of testing with the Na_2CO_3, until finally a yellow color appears. This proves that the mercuric nitrate has been added in excess,—consumed all the urea in combination and left some mercuric nitrate to react with the sodic carbonate, which it does by forming sodic nitrate and the yellow oxide of mercury.

If now the mercuric solution is correct it will require exactly 20 c.c. of it to precipitate the whole of the urea in the 10 c.c. of the standard solution of urea, and enough more to react with the sodium carbonate. If the yellow coloration does not occur under these circumstances, there has been some impurity in the mercury compound employed, or some error in making up the solution.

Process.—Take 40 c.c. urine and 20 c.c. of the baryta solution, and throw them into a beaker-glass. By this means the phosphates, sulphates, and carbonates are precipitated. They are removed by filtration through a *dry* filter, and if the filtrate happen not to be quite clear, it may be passed through a second time.*

While this is taking place, the burette is filled to 0 with the mercuric nitrate solution, and 15 c.c. of the filtrate from the mixed baryta fluid and urine, containing of course 10 c.c. of pure urine, are measured off into a small beaker-glass. Into this the mercuric nitrate solution is allowed to fall from the burette, first, a number of cubic centimeters approaching the last two figures of the specific gravity (that is, if the specific gravity is 1017, drop say 15 c.c.)

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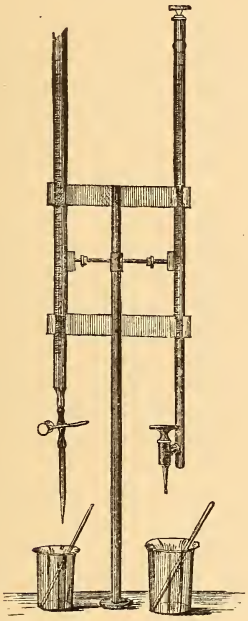
* If the filtrate is not alkaline, the precipitation of the phosphates and sulphates may not have been complete. This may be determined by adding a drop or two of the baryta mixture to the filtrate, when, if a precipitate appears, a fresh quantity of urine must be taken, and a larger proportion of the baryta solution added.

before testing with the sodium solution. If no yellow coloration appears on such testing, then proceed cautiously, adding a fractional part of a cubic centimeter at a time, and testing with the Na_2CO_3 until the yellow coloration is

FIG. 14.



Burette stand with two forms of burette.

obtained. When that point is reached, read off the number of cubic centimeters employed.* The number of

* The tinge of yellow at which we cease the titration must of course be the same as that at which in originally standardizing the nitrate of

cubic centimeters of mercury solution thus used, minus 2, multiplied by .010 gram, gives the amount of urea in fractions of a gram contained in 10 c.c. of the urine, when the latter is of average composition,—that is, when it contains no abnormal constituent, and the amount of chlorides is nearly normal.

Corrections Explained.—*The two cubic centimeters are first subtracted because it takes about this quantity of the reagent to convert the chloride of sodium into the nitrate*, and until this combination is complete, the combination with the urea does not begin. Hence this amount must first be subtracted.

If, however, the chlorides are not of average amount, but diminished or increased, and we wish to be accurate, we must first estimate the amount of chlorides calculated as NaCl in 10 c.c. of the urine, by the process to be explained under chlorides, and from a fresh quantity of urine remove the whole of the chlorides by a standard solution of silver nitrate. For this purpose a solution of nitrate of silver is required of such strength that 1 c.c. will precipitate 10 milligrams sodium chloride. 29.059 grams of fused nitrate of silver, dissolved in distilled water and diluted to a liter, will be such a fluid.

In 10 c.c. of the original urine we determine with the nitrate of silver solution the chloride of sodium by the method for the determination of the chlorides, p. 165. Suppose there are required for this 17.5 c.c. of the silver solution, this indicates 175 milligrams sodium chloride.

Take now 30 c.c. (containing 20 c.c. of urine) of the filtrate from the mixture of baryta fluid and urine, add a drop of nitric acid, and then 17.5×2 c.c. $= 35$ c.c. of the nitrate of silver solu-

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mercury solution the titration was stopped. It is evident that ceasing the titration, now at a slight tinge and again at a marked yellow coloration, must give rise to an error, which practice will soon teach the student to avoid.

tion. This will precipitate all the chlorides, which should be removed by filtration, and the filtrate may be now estimated for urea. It is important always to bear in mind the exact amount of urine operated with after adding the nitrate of silver solution to a mixture of baryta solution and urine, of which only two-thirds are urine. Thus, if 35 c.c. of the silver solution are added to 30 c.c. of the filtered mixture of urine and baryta fluid, of the resulting 65 c.c. only 20 would be urine minus the chlorine, or out of 32.5 c.c. 10 would be urine minus the chlorine.

If the case be one of inflammation, as pneumonia, where there is a total or almost total absence of chlorides, they may be thrown out of the question altogether.

Further Corrections.—If the number of cubic centimeters of mercury solution added to 15 c.c. of the mixture of urine and baryta fluid exceeds 30—that is, if the amount of urea in the unmixed urine exceeds 3 per cent.—we must, for the number of c.c. of the mercurial solution required above 30, add half the number of cubic centimeters of water to the urine mixture and make a second titration. Thus, suppose 36 c.c. are required on the first titration, the excess is 6 c.c., therefore 3 c.c. of water must be added to the mixture before making the second titration.

If the unmixed urine contains less than 3 per cent. of urea, then for every 4 c.c. of the test solution used below 30 there should be deducted 1 c.c. from the entire number of cubic centimeters of the mercurial solution used.

These corrections are rendered necessary by the fact that in standardizing the reagent it was mixed with just half its volume of the urea solution, conditions which we have (in regard to dilution) when 10 c.c. of urine containing 3 per cent. of urea are mixed with 5 c.c. baryta mixture and 30 c.c. of the mercury solution. Hence, any excess of reagent employed above 30 c.c. for 15 c.c. of the urine mixture should be diluted with half its volume of water to reduce such excess to the same degree of dilution as was present in standardizing the reagent. So, on the other hand, if less than 30 c.c. of the reagent are required, that employed will be under greater dilution than was present in standardizing the reagents; hence, the reduction mentioned above.

Estimation of Urea by the Hypobromite Process.—The principle on which this process is based—that urea, when brought in contact with hypochlorite of calcium, is decomposed into nitrogen, carbonic anhydride and water—was suggested many years ago by Davy,* and at the same time by LeConte.† In 1874, Messrs. Russell and West‡ again directed attention to the subject, substituting an alkaline solution of hypobromite of sodium and caustic soda, which yields similar products; the carbonic anhydride being absorbed by the caustic alkali. The following is the reaction:

$$CON_2H_4 + 3(NaBrO) = 3(NaBr) + CO_2 + 2H_2O + N_2;$$

the volume of nitrogen disengaged being the measure of the urea.

Many forms of apparatus have been suggested by different experimenters, all based upon the assumption that one gram of urea contains 372 c.c. nitrogen, measured at 0° C. and 760 mm. barometric pressure; or that each c.c. of nitrogen evolved, measured under the conditions stated, represents 0.00282 gram urea.§

The simple apparatus shown in Fig. 15., devised by Dr. John Marshall,|| is that in use in the chemical laboratory of the University of Pennsylvania. It consists of a graduated measuring tube, a graduated pipette, a funnel tube and a saucer-like apparatus which serves as a stand and also as a receptacle for the hypobromite solution which in the operation overflows from the measuring tube. The

* Philosoph. Mag., 1854, p. 345.

† Comptes Rendus, xlvii., 237.

‡ Journal of the Chemical Society (London), August, 1874.

§ See note to p. 156.

|| Zeit. f. Physiolog. Chemie. Bd. xi. p. 179.

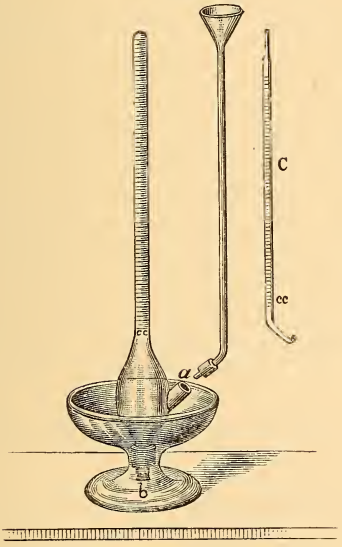
measuring tube, including the bulbous part, holds about 77 c.c., and can, by means of a perforated cork, be fastened to the saucer-like vessel. The apparatus can easily be taken apart and cleansed of the grease-like material which, in the performance of the hypobromite method, usually accumulates in the measuring tube.

The alkaline hypobromite solution is made by dissolving 100 grams of caustic soda in 250 c.c. of water, and adding 25 c.c. of bromine to the solution thus produced.

$$2NaHO + Br_2 = NaBr + H_2O + NaBrO.$$

Process.—The thumb is placed over the opening *a*, and the hypobromite solution is poured in through *b*. The latter opening,

FIG. 15.



b, is then closed by a rubber stopper, and any air bubbles in the tube are allowed to pass out at the opening *a*. The graduated

tube is now reversed and the closed oval end of the tube is fastened in the opening in the saucer-like vessel. A measured quantity of urine (one cubic centimeter generally suffices) is allowed to pass from the pipette C into the hypobromite solution through the opening *a*. Decomposition of the urea immediately occurs, and the evolved nitrogen collects in the upper part of the graduated tube. The carbon dioxide evolved in the decomposition of the urea is absorbed by the excess of caustic soda in the hypobromite solution. About 20 minutes are required for the complete decomposition of the urea. When the decomposition is completed and all the gas bubbles have collected with the gas in the upper part of the tube, the atmospheric pressure is equalized by attaching the funnel tube (*d'*) to the opening (*a*) and pouring into it hypobromite solution or water until the surface of the liquid in both tubes stands at the same level. The number of cubic centimeters of nitrogen, the temperature and the barometric pressure are read off, and the percentage of urea calculated by the following formula:

$$p = \frac{100\ v.\ (b - b')}{760 \cdot 354.33\ a.\ (1 + 0.00366\ t.)}$$

In which

p represents the weight of urea in grams in 100 c.c. of the urine.
a " the volume of urine employed.
b " the observed barometric pressure in millimeters.
b′ " the tension of aqueous vapor for the temperature t.
t " the observed temperature (Centigrade).
v " the volume of nitrogen, in cubic centimeters, obtained.

For clinical purposes the thermometer and barometer may be ignored, and the reading in centimeters need only be multiplied by 0.00282 * gram, representing the weight of urea corresponding to

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* The amount of nitrogen gas which, theoretically, should be evolved from 1 gram of urea is 372.7 c.c., but Hüfner has† shown that practically by the hypobromite method only 354.33 c.c are evolved, and, therefore, 1 c.c. of the nitrogen at 0°C and 760 m.m. pressure corresponds to 0.00282 gram of urea. Hence the latter figures are used in the calculation. If the full theoretical quantity of nitrogen were

† Zeit. f. Physiolog. Chemie, Bd. 1, p. 350.

a single c.c. of nitrogen gas. Thus, suppose 9.6 c.c. of gas have been read off. Then the 1 c.c. of urine (or as much as was used in the operation) must have contained 0.00282 × 9.6 = 0.027 gram, whence can be calculated either the percentage or 24 hours' quantity of urea.

An apparatus for this purpose has also been devised by Dr. William H. Greene,* of Philadelphia, and another by Dr. Charles A. Doremus,† of New York, and furnished by Eimer & Amend, of that city.

In experiments made by Messrs. West and Russell, Mr. Richard Apjohn, Dr. Dupré, Dr. M. Simpson, Mr. C. O'Keefe, Prof. Theodore G. Wormley, and others, with solutions containing known quantities of urea, astonishingly accurate results were obtained, quite sufficiently so for clinical purposes.

M. Depaine (*Journ. de Pharm. d'Auv.*, 1877) recommends that 4.5 per cent. be deducted from the total amount of urea found, to eliminate the error caused by the simultaneous decomposition of uric acid and creatinin.

It is generally acknowledged that not quite all the nitrogen of the urea in a given solution is liberated by the hypobromite process, but authorities are not all agreed as to the quantity retained. M. Leconte (*Chem. Gaz.*, 1858) obtained .92; Foster announced (*Journ. Chem. Soc.*, March, 1879) that .92 was obtained; Russell and West obtained .94; others secure a larger proportion, as much as .95; others a still different proportion. Mehu (*Journ. Chem. Soc.*, Nov., 1879), admit-

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evolved in the practical application of the method, then 1 c.c. of the nitrogen, at 0° C and 760 mm. pressure, would be equal to 0.00268 grm. of urea.

* First described in the Philada. Med. Times for January 12, 1884, p. 278.

† Medical News, May 30, 1885.

ing that about .08 of nitrogen is retained, first suggested that this deficiency is overcome if a solution of either cane or grape sugar is mixed with the urine and hypobromite solution, the small amount of nitrogen otherwise retained being now liberated. But a little later Esbach (*Journ. Chem. Soc.*, December, 1879) and M. Jay (*Bul. Soc. Chim.*, 1880) announced that a solution of glucose alone mixed with the hypobromite solution evolves a gas. Fauconnier (*Bul. Soc. Chimique*, February, 1880) then announced that with a solution of *glucose*, the theoretical quantity of nitrogen is evolved from urea solutions by the hypobromite, but with a solution of *cane sugar* only .94. Again, Jay's experiments (*Ibid.*) go to show that if a solution of glucose is mixed with the hypobromite and urea solution, no additional gas is evolved in the short time allotted to an ordinary estimation, while with cane-sugar solutions an appreciable amount is evolved. But as it is practically almost impossible to obtain pure glucose, it resolves itself into this, that neither the glucose nor cane sugar solution should be mixed with the hypobromite solution and urine. My colleague, Professor Wormley, in some recent experiments in connection with this subject, has made an important observation, which may serve to explain some of the discrepancies in the results obtained by the observers above alluded to. He finds that on mixing solutions of sugar and hypobromite *a large amount of heat is evolved*, as much as 8.9° C. or 16° F. with sugar cane and 14.5° C. or 26.2° F. with grape sugar. This, causing an expansion of the gas, would permit a larger amount of nitrogen to be read off.

Fowler's Hypochlorite Process for Urea.*—This method is based upon the fact that there is a difference in the specific gravity of urine before and after the decomposition of its urea by the hypochlorites; and that such difference bears a definite relation to the quantity of urea present. Dr. Fowler found that every degree of density

* Fowler, Prize Essay to the Alumni Association of the College of Physicians and Surgeons, New York. Published in the New York Medical Journal, July, 1877.

lost corresponds to .77 of 1 per cent., or about 3½ grains per fluidounce. The hypochlorite solution employed is Squibb's solution of chlorinated soda, or Labarraque's solution, of which seven parts will destroy the urea in one part of urine, unless the amount is very large, in which event the urine should be diluted by an equal bulk of water, and the result multiplied by 2.

Process.—1st. Add to 1 volume of the urine 7 volumes of the hypochlorite solution. Effervescence due to the liberation of nitrogen will immediately take place. Shake the jar containing the mixture occasionally, and stand it aside for two hours, when the urea will have been decomposed. Now take the specific gravity of the quiescent fluid.

2d. Ascertain the specific gravity of the mixed urine and hypochlorite solution before decomposition. To do this, multiply the specific gravity of the pure hypochlorite solution by 7, add this to the specific gravity of the pure urine and divide by 8. The result is the specific gravity of the mixed fluid. From this subtract the specific gravity of the quiescent mixture after decomposition of the urea, multiply the difference by .77, and the result is the percentage of urea.

As changes of temperature affect the specific gravity and volume of liquids, the hypochlorite solution and urine should be mixed and the jar set aside along with a bottle of the urine and the hypochlorite solution in the same place, subject to the same temperature. When decomposition is complete, the specific gravities can be taken and the calculation made.

Example.—Suppose the specific gravity of the urine is 1010, and that of the hypochlorite solution 1045, that of the mixed fluid will be

$$\frac{1045 \times 7 + 1010}{8} = 1040.$$

Now suppose the specific gravity of the decomposed fluid is 1038, then $(1040-1038) \times .77 = 1.54$ the percentage of urea.

This very simple and easy process has been found quite accurate, and is not interfered with by sugar or albumin.

XVI. Uric Acid ($C_5H_4N_4O_3$).

When uric acid is spoken of as a constituent of normal urine, it is never to its free state that allusion is made, but to its combinations, chiefly with potassium, sodium, and ammonium, but also with calcium and magnesium, usually known as mixed urates. Uric acid itself is so extremely insoluble (one part requiring 14,000 of cold and 1800 of hot water to dissolve it) that it is immediately precipitated on being freed of its bases. In quantity it is found ranging .4 to .8 gram (6.17 to 12.34 grs.) in the twenty-four hours, in health varying *pari passu* with urea, of which it is a stage short in oxidation.

Detection by the Microscope.—Its presence as such is recognized by the microscopic peculiarities of its crystals, which in their typical form may be said to be "lozenge-shaped," or, as described by the Germans, "whet-stone-shaped." They are, moreover, always colored yellowish-red, being with their salts the only urinary deposits thus stained, so that when a sediment is seen of which the elements are thus colored, it may, without hesitation, be put down as composed of uric acid or its combinations. More will be said of these crystals in treating of sediments, where their discussion more properly belongs.

The Murexid Test.—The murexid test for uric acid and its combinations is one of extreme beauty. A small portion of sediment, or the residue after evaporation, is placed on a porcelain plate or piece of platinum, a drop or two of nitric acid added to dissolve it, and the solution carefully evaporated over a spirit lamp flame. When dry, a drop or two of liquor ammoniæ is added, when there promptly appears a beautiful purple color, which will gradually diffuse itself as the ammonia spreads. The

murexid reaction is believed to depend upon alloxan, alloxantin, and ammonia, which arise under the action of the hot nitric acid. This reaction is also said to occur with tyrosin, hypoxanthin, and xanthoglobulin, and Schiff accordingly recommends the—

Carbonate of Silver Test for Uric Acid.—This is very delicate, and is most conveniently applied as recommended by Harley. Dissolve a little uric acid in a solution of sodium or potassium carbonate, place a drop or two of the solution on paper, and add a solution of nitrate of silver. A distinct gray stain promptly occurring indicates the presence of uric acid. Neither of the tests, however, discriminates between uric acid and urates. The microscope most easily does this.

Quantitative Estimation of Uric Acid.—To 200 c.c. add 20 c.c. of hydrochloric or nitric acid, and set aside in a cool place, as a cellar, for twenty-four hours. At the end of that time the uric acid crystals, highly colored, will be found adhering to the sides and at the bottom of the beaker. Collect the uric acid on a weighed filter, wash thoroughly with distilled water. Dry the filter and uric acid at a temperature of 100° C. (212° F.), weight, and the weight of the two, minus the weight of the filter, will be the weight of the uric acid in 200 c.c., except the small portion retained in the acid and washings. Neubauer advises to add to the result 0.0038 gram uric acid for every 100 c.c. of these fluids.

XVII. Urates.

It has already been said that in health, practically all the uric acid of the urine is held in combination with potassium, ammonium, sodium, calcium and magnesium, of

which, according to Bence Jones, those with potassium and ammonium are most abundant. These are very soluble compounds at the temperature of the body, but are precipitated in amorphous granules when the temperature of the urine is lowered, as in winter weather.

Their physiological and pathological significance depends altogether upon the uric acid they contain, but there are some points of reaction with which the student should be quite familiar. These grow out of the fact that uric acid is a bibasic acid, forming neutral and acid salts, and that *the acid salts are much less soluble than the neutral*, requiring 124 parts of boiling and 1120 parts of cold water for their solution. They form, therefore, the bulk of urate deposits, while urates, which remain in solution after such reduction of temperature as constantly takes place in an apartment, must be, if not neutral, at least less acid than those which form the sediment. And a solution remaining for some time clear under such circumstances must contain urates of sodium, etc., with a large proportion of the alkaline base.

The practical application of this fact is seen in this, that when an acid is added to such solution of neutral urate, by seizing upon a portion of the base, it leaves an *acid* urate of sodium, which, in consequence of its relative insolubility, is promptly precipitated in a finely *granular* form producing a decided opacity. Now, this is precisely what often happens in the nitric acid test for albumin. The urine is highly charged with neutral urates which are held in solution. Nitric acid is added, and down goes a precipitate, not crystalline, but *amorphous*, which is composed of acid urate of sodium. And if Heller's method is followed, an opaque zone is formed at the point of contact

between the acid and urine, which may be mistaken for albumin, but which, besides presenting certain visual characters of its own, which have been described, p. 40, is readily soluble by heat. If urine presenting this reaction with acid be allowed to stand for some time, the milky opacity gradually passes away, and is substituted by a very small crystalline sediment of uric acid. By longer action of the acid the remainder of the base is entirely withdrawn, leaving the free acid, which is deposited in crystals. It has already been stated that this precipitate by nitric acid is considered by Thudichum to be not acid urates, but *hydrated uric acid.*

The remaining organic constituents of urine, *creatinin*, *xanthin*, *hippuric acid*, *oxalic acid*, *lactic acid*, and *phenylic acid*, having little practical significance as such, require only to be mentioned in this connection.

Mucus and the crystalline combination of *oxalic acid* with lime will be further considered in treating of sediments.

Hippuric Acid is interesting in forming one of the most striking connecting links between the urine of carnivora, omnivora, and herbivora, replacing in the last the uric acid of the first, while in man, who consumes a mixed diet, we have both uric acid and hippuric, that is, an intermediate state. But while hippuric acid is increased in man by a vegetable diet, it is not wholly absent with animal food. It is increased in diabetes, where also it almost replaces uric acid. If 10 grains benzoic acid be taken in the evening, the next morning crystals of hippuric acid will usually be found in the urine. The typical form of these is a four-sided prism, with two or four beveled surfaces at its ends, but from this there are deviations. In the twenty-four hours' urine of man, .5 to 1 gram (7.7 to 15.4 grs.) is separated.

Inorganic Constituents.

XVIII. The Chlorides.

The chlorides found in the urine are chiefly those of sodium, with a small proportion of chloride of potassium and ammonium.

In health the chlorides of the urine are almost an exact measure of the same substances taken in with the food, and amount to 10–16 grams (154.3 to 246.8 grs.) in the twenty-four hours.

Detection and Approximate Estimation.—If a drop of urine be slowly evaporated on a glass slide, characteristic octahedral crystals and rhombic plates of a combination of urea and chlorine make their appearance, and may be examined by the microscope. But more available for detection and approximate estimation is

The Nitrate of Silver Test.—Nitrate of silver in solution throws down both the phosphates and chlorides from urine. But if a few drops of nitric acid be first added, the phosphates will be held in solution, and only the chlorides will fall as opaque white chloride of silver.

From normal urine containing ½ to 1 per cent. of chlorides, they are precipitated by a *single* drop of a solution of nitrate of silver, 1 part to 8, in cheesy lumps, which do not further divide themselves, or make the urine more milky by moving the glass about. *If, however, the chlorides are diminished* to $\frac{1}{10}$th per cent. or less, the addition of a single drop of the silver solution no longer produces the white cheesy lumps, but a simple cloudiness, and the entire fluid appears equally milky. If, finally, there should be no precipitate whatever, then the chlorides are totally absent.

The presence of albumin in moderate amount does not interfere with the test, but if abundant it must be removed.

Clinical Significance.—The chlorides are diminished in all febrile conditions, whether of local or general origin, except, it is said, intermittent fever. Especially is this the case where there are any exudations, solid or fluid, in which the chlorides seem to be eliminated. In acute pneumonia, where they are often totally absent from the urine, they appear abundantly in the saliva. In this affection, and indeed in all acute diseases, their disappearance from the urine indicates an increment in the disease, and their reappearance an improvement. In pneumonia a decline in the disease may often be detected through their return before physical or any other signs point to improvement. Hence a daily trial of the urine for chlorides becomes important.

Volumetric Process for the Chlorides.

Of several volumetric processes employed for the estimation of chlorides, **Mohr's nitrate of silver method** is here given. There are required—

1. A cold saturated solution of neutral chromate of potassium.

2. A solution of nitrate of silver, such that 1 c.c. = 10 milligrams NaCl. This is made by dissolving 29.075 grams pure fused nitrate of silver in distilled water and diluting to a liter.

Process.—Put 10 c.c. of the urine into a platinum crucible, dissolve in it 1 or 2 grams potassium nitrate, free from chlorides, and evaporate the whole slowly to dryness. Expose the remainder first to a gentle and afterwards to a strong heat until the carbon is completely oxidized, and

the residue a white molten saline mass. The entire white mass is then dissolved in a little water, placed in a beaker-glass, and the platinum capsule washed off into it with the wash-bottle. Dilute nitric acid is then carefully dropped into the alkaline fluid until it is faintly acid, a small pinch of calcium carbonate is introduced to make it neutral, and the excess of lime filtered off. To the mixture 2 or 3 drops of the potassium chromate solution are now added, and the silver solution allowed to flow in from the burette while stirring the mixture, until a distinct red color remains. The color continues canary-yellow until all the chlorides are decomposed. As each drop falls into the urine, it must be carefully watched for the least tinge of red surrounding the precipitate of chloride of silver; the very next drop after the complete decomposition of the chlorides gives a permanent red color, due to the presence of silver chromate. The number of cubic centimeters consumed $\times$ .010 gram will give the amount of chlorides, estimated as NaCl, in 10 c.c. urine, whence the total is calculated.

XIX. Phosphates.

The phosphates of the urine are composed partly of *earthy* and partly of *alkaline* phosphates. The former are insoluble in water, but soluble in acids; they are held in solution in acid urine by free carbonic acid, and are precipitable from it by alkalies. The *alkaline* phosphates are soluble in water, and are not precipitated from solution by alkalies.

(*a*) The **earthy phosphates** are *phosphates* of *calcium* and *magnesium*, and are contained in urine in but small quantities—1 to 1.5 gram (15.43 to 23.14 grains) in twenty-four hours. The proportion of calcium to the magnesium phosphate is as 33 to 67.

Detection and Approximate Estimation.—The presence of the earthy phosphates is shown by adding any alkali, as caustic ammonia or potash.

Their quantity may be *approximately* estimated in the following simple way, directed by Hoffmann and Ultzmann. A test-tube, 16 centimeters (6.2992 inches) long and 2 centimeters (.787 inch) wide, is filled *one-third* with clear or filtered urine, to which a few drops of caustic ammonia or caustic potash solution are added, and warmed gently over a spirit-lamp until the earthy phosphates begin to separate in flakes. It is then placed aside for ten or fifteen minutes for them to subside. If the layer of sediment is one centimeter (.3937 inch) high, the earthy phosphates are present in normal amount; if they occupy 2 to 3 centimeters (.787 to 1.181 inch), they are increased; if, on the other hand, only a few flakes are visible, the earthy phosphates are diminished.

Further, in normal urine the earthy phosphates are precipitated white, but if the urine contains abnormal coloring matter, they fall variously colored. If the urine contains blood-coloring matter, the earthy phosphates appear blood-red or dichroic; if there be present vegetable coloring matters, as rhubarb, senna, etc., they are colored rosy-red to blood-red, and by the biliary coloring matters yellowish-brown, and by uroerythrin, gray.

The earthy phosphates are deposited from alkaline urine, and a most important precaution must here be observed not to make such a *deposit* for an excess of phosphates. The phosphates may really be *diminished*, and yet, in consequence of the reaction of the urine, a copious *deposit* may be present. The possible *precipitation of earthy phosphates by heat alone*, as a source of error in testing for albumin,

has already been alluded to. This frequently occurs, and is best explained on the supposition of Dr. Brett that the earthy phosphates are held in solution in urine by carbonic acid, which, being dissipated by heat, allows the phosphates to fall. It should be further stated, however, that Dr. Owen Rees believes the phosphates are held in solution by ammonium chloride, which would also be dissipated by heat.

Clinical Significance.—The earthy phosphates are increased in the urine by diseases of the bones, especially if extensive, as in osteomalacia and rickets, in chronic rheumatoid arthritis, in diseases of the nerve-centres, and after great mental strain; but especially are the earthy phosphates increased by the food and drink, some contending that all variations in the earthy phosphates are due to this cause. In renal diseases, on the other hand, the phosphates are said to be diminished. Earthy phosphates are often found deposited in conditions of dyspepsia and overwork, but this may generally be traced to changes in the reaction of the urine.

(*b*) The **alkaline phosphates**, soluble in water and not precipitated by ammonia or alkalies, form the chief bulk of the phosphates, averaging, according to Breed, 4 grams (61.72 grains) in the twenty-four hours, though Neubauer, by volumetric analysis, has seldom found more than two grams (30.86 grains) in this period. Four grams correspond to two grams phosphoric acid. They are almost wholly made up of *acid sodium phosphate*, with possible traces of *potassium phosphate*. The acid sodium phosphate was believed by Liebig to be the cause of the acid reaction of the urine.

Approximate Estimation of Alkaline Phosphates.—Accurately to estimate the alkaline phosphates, it is

necessary, first, to remove the earthy phosphates, which is easily done by precipitating them with ammonia and filtering them out. For approximate estimation, however, this is not necessary, since they are in the first place present in comparatively small quantity, and, secondly, do not vary much in disease. Practically, therefore, they are disregarded, and to a suitable quantity of urine placed in a beaker-glass about *one-third* as much of the magnesian fluid (p. 16) is added. *All* of the phosphates are thrown down in the shape of a snow-white deposit. If the entire fluid present a *milk-like cloudy appearance*, the alkaline phosphates may be considered present in normal amount; if it is denser, more cream-like, there is an increase. If, on the other hand, the fluid is but slightly cloudy, transmitting light distinctly, the phosphates are diminished.

Nitrate of Silver Test.—A solution of nitrate of silver added to urine throws down a yellow precipitate of phosphate of silver, and chloride of silver. Both are soluble in *ammonia*, the silver phosphate also in nitric acid, but not the chloride. If, therefore, a few drops of ammonia be added, they will promptly disappear. If now nitric acid, just sufficient to neutralize the ammonia, be added, the precipitate will again appear; but the moment the nitric acid is present in excess, the silver phosphate is redissolved, but the chloride remains in suspension. If now enough ammonia be added again to neutralize the nitric acid, the phosphate of silver will again fall; but if an excess be added, the entire precipitate, including the chlorides, will be redissolved.

Clinical Significance of Alkaline Phosphates.—The alkaline phosphates in the urine are influenced chiefly by the food, whence they are mainly derived; phosphorus

is also oxidized in the economy, and a small part of the phosphates is doubtless derived from the disintegration of nervous and muscular tissues. Any increased activity of vital processes, as inflammations and fevers, would, therefore, favor their increase.

Phosphatic Diabetes.—Increased attention has been recently called to the elimination of phosphates by the researches of Prof. Teissier, of Lyons, and Dr. Charles Henry Ralfe, of London. The former first described a condition which he called phosphatic diabetes, in which there is a continuous and successive discharge of phosphates in the urine attended with symptoms not unlike those of saccharine diabetes. Of the cases of this condition Teissier makes four groups. 1st. It may be observed in certain functional derangements of the nervous system. 2d. It may precede or accompany certain affections of the lungs. 3d. It may coexist with glycosuria or alternate with it. 4th. It may run a distinct course of itself. Of 13 cases described by Ralfe, all except 2 occurred in male adults. The symptoms common to all, although varying in degree, were loss of flesh, aching rheumatic pains, chiefly of the lower back and pelvic regions, a dry, harsh skin, a tendency to boils, appetite generally ravenous, but in some cases a morbid refusal of food.

Volumetric Process for Phosphoric Acid.

This process is based upon the facts that,

1. When a solution of phosphate acidulated with acetic acid is treated with a solution of nitrate or acetate of uranium, a *precipitate* falls which is composed of uranium phosphate.

2. When a *soluble* salt of uranium is added to a solution

of potassium ferrocyanide, a reddish-brown precipitate or color is developed.

The solutions required are,

1. A standard solution of sodium phosphate, made by dissolving 10.085 grains of well-crystallized sodium phosphate ($Na_2HPO_4 + 12H_2O$) in distilled water, and diluted to a liter; 50 c.c. then contain .1 gram P_2O_5.

2. Saturated solution of potassium ferrocyanide.

3. Sodium acetate solution, made by dissolving 100 grams sodium acetate in 100 c.c. pure acetic acid, and diluting with distilled water to 1000 c.c.

4. Solution of uranium acetate, such that 1 c.c. will correspond to .005 gram or 5 milligrams phosphoric acid.

To prepare the Uranium Acetate Solution.—Dissolve 20.3 grams of yellow uranic oxide in strong acetic acid previously diluted with distilled water to nearly a liter. To determine the strength of this solution, place 50 c.c. of the standard solution of sodium phosphate in a beaker with 5 c.c. of the solution of sodium acetate, and heat in a water-bath to 90° to 100° C. (194° to 212° F.). The uranium solution is then allowed to run from a burette into the warm mixture until precipitation ceases. Then a drop of the mixture is carried by a glass rod into contact with a drop of the ferrocyanide of potassium solution on a white plate, or to a piece of the filtering-paper impregnated with it. If the reddish-brown of the uranium ferrocyanide does not appear, continue the cautious addition of the uranium solution until the color responds to the test. The quantity used is then read off, being that which is sufficient to decompose sodium phosphate corresponding to .1 gram of P_2O_5, whence is calculated the amount of distilled water to be added to make 1 c.c. correspond to .005 gram of phosphoric acid.

Process.—Take 50 c.c. of urine, add 5 c.c. of the sodium acetate solution, and warm in a water-bath as above. Fill the burette with the uranium solution, and drop it into

the mixture while warm, testing with the ferrocyanide solution. The number of cubic centimeters used multiplied by .005 will give the phosphoric acid in the 50 c.c. of urine, whence calculate the quantity for the twenty-four hours.

XX. Sulphates.

The sulphates found in the urine are those of sodium and potassium, the former preponderating. The quantity in twenty-four hours is three to four grams (46.29 to 61.72 grains), corresponding to 2 grams (30.86) sulphuric acid.

Detection and Approximate Estimation.—This is simple with any of the barium compounds, which throw down a white precipitate of barium sulphate. A little acid, as hydrochloric, should previously be added, in order to hold in solution the barium *phosphate*, which is otherwise thrown down, or the acid may be previously added to a solution of barium chloride.

If to a small quantity of urine in a beaker-glass one-third as much of the acidulated solution of barium chloride (1 part to 8 plus ½ a part hydrochloric acid) is added, and there occurs an *opaque* milky cloudiness, the proportion of sulphates is normal; if the opacity is intense, and the whole mixture has the appearance and consistence of cream, the sulphates are increased; if, on the other hand, there is only a slight cloudiness, so that light is still transmitted, the sulphates are diminished.

Clinical Significance.—The sulphates are derived partly from the food and partly from the tissues, are increased by the introduction of sulphur compounds, sulphuric acid and its soluble combinations, by animal food, and by any causes producing increased rapidity of tissue change; as active exercise, the inhalation of oxygen,

by febrile movements, and fevers. The greatest increase has been observed in meningitis, cerebritis, rheumatism, and affections of the muscular system. They are diminished in an exclusively vegetable diet.

The Volumetric Process for Sulphuric Acid.

This depends upon the principle that a solution of chloride of barium will throw down a precipitate from a given quantity of urine, so long as any sulphuric acid is present; and further, that in thus treating a specimen of urine acidulated with HCl, a neutral point is reached at which the filtrate will show a slight opacity as well with the sulphuric acid as with the barium chloride solution. In such a fluid we are to suppose potassium chloride, barium chloride, and potassium sulphate balancing each other. If now either barium chloride or potassium sulphate is added, it itself is decomposed, and barium sulphate precipitated.

The solutions required are—

1. Solution of barium chloride so concentrated that 1 c.c. will precipitate exactly 12.25 milligrams H_2SO_4, or 10 milligrams SO_3—prepared by dissolving 30.5 grams dry crystallized chloride of barium, and diluted to a liter.

2. Solution of potassium sulphate, such that 1 c.c. = 12.25 milligrams H_2SO_4, or 10 milligrams SO_3; prepared by dissolving 21.775 grams chemically pure powdered potassium sulphate, dried at 100° C. (212° F.), and diluting to a liter.

Process.—Place 100 c.c. urine, acidulated with 20 to 30 drops hydrochloric acid, and heat it in a water-bath. When boiling, allow 5–8 c.c. of the barium solution to flow in from a burette. Remove the heat and allow the precipitate to subside. If the fluid becomes rapidly clear,

allow another cubic centimeter or two of the barium solution to flow in, reapply the heat, and filter 10 to 12 drops of the urine into a small test-tube, add some of the barium solution, and observe whether there is a precipitate or not. If not, add to another portion a few drops of the potassium sulphate solution, by which we learn whether an excess of the barium solution has been added or not. If, however, the barium solution still produces a precipitate in the portion removed for testing, the latter is returned to the beaker, and more solution allowed to flow in, determining the quantity somewhat by the intensity of the reaction in the test-tube, and the process repeated until no precipitation takes place with the barium, and until a *slight* cloudiness takes place when adding the potassium sulphate to a portion of the filtered mixture. If the latter is an intense reaction, say at 12 c.c., then we know that the correct point is somewhere between 11 and 12, and the process is repeated as far as 11 c.c., when it is continued very cautiously, adding only fractions—$\frac{1}{10}$th of a centimeter—until the right point is reached, whence the calculation is made as before.

PART II.

URINARY DEPOSITS.

Preliminary Remarks—Secondary Deposits.

It has already been said that strictly normal freshly passed urine, of *acid* reaction, contains no sediment whatever, except the faint flocculi of mucus which gradually subside towards the bottom, and entangle a few mucus corpuscles and an occasional epithelial cell. Should the urine, however, be alkaline, as is frequently the case three or four hours after a meal, it may be more or less cloudy at the moment it is passed, and quickly deposit a flocculent precipitate of *earthy* phosphates, which may occupy considerable bulk. They will be found by microscopic examination to be made up of amorphous granules, and will quickly disappear on the addition of a few drops of any acid.

But even urine which is strictly normal will, in the course of time, form deposits as the result of changed reaction. These deposits differ with the stages of such reaction, and should be perfectly understood by the student before he is ready to interpret any sediment arising from other causes.

1. After normal urine, completely without sediment, has stood for a time, especially at a moderate temperature, there is often observed a precipitate of amorphous granular

matter, readily soluble by heat, which is made up of acid urates of potassium, sodium, and ammonium, with which urates of lime and magnesium are occasionally commingled. (See lower portion of Fig. 16.) A little later they are replaced by rhombic crystals of uric acid, stained yellowish or yellowish-red. These are often associated with octahedral crystals of the oxalate of lime.

The explanation given by Scherer of the occurrence of these deposits is that of the so-called *acid* fermentation, in which, through the agency of the mucus of the bladder, acting as a ferment, *lactic* and *acetic* acids are formed out of the coloring-matters. These take away a part of the base from the neutral or alkaline urates, and produce first the more insoluble acid urates named above, which are deposited; later they combine with the remainder of the base also, and leave the crystalline uric acid sediment.

As though favoring this so-called acid fermentation, there are also often found at this stage in urine spores of *torula cerevisiæ*—the yeast fungus; small, oval, transparent, structureless cells, to be again referred to. Sufficient proof that such fermentation takes place is, however, wanting.

A much more satisfactory explanation of the occurrence of these deposits has been offered by Voit and Hoffmann,* who attribute the decomposition of the basic urates to the acid phosphate of sodium, the excess of phosphoric acid playing the part of the acetic and lactic acid in the fermentation theory, and decomposing the alkaline urates in the same way and with the same results. They prove their position by an artificial production of the same results by

* Neubauer and Vogel, Analyse des Harns, vi Aufl., 1872, p. 113, from Zeitschrift für Analyt. Chemie, Bd. 7, p. 397.

adding a solution of acid phosphate of sodium to a solution of basic urates. The extent to which the reaction goes will depend upon the quantity of acid phosphate of sodium present and the length of time during which the reaction has been permitted to proceed. It is possible also for the latter to begin at the moment of secretion, and to continue in the bladder, causing deposits of acid urates and uric acid to appear as "gravel" or "sand" immediately after the urine is passed. Such a condition would be pathological. According to these authors, a more rapid action of the acid sodium phosphate produces an amorphous precipitate, and a slower separates the crystalline uric acid. The more rapid reaction may be induced by a more abundant separation of the acid sodium phosphate or a greater concentration of the urine.

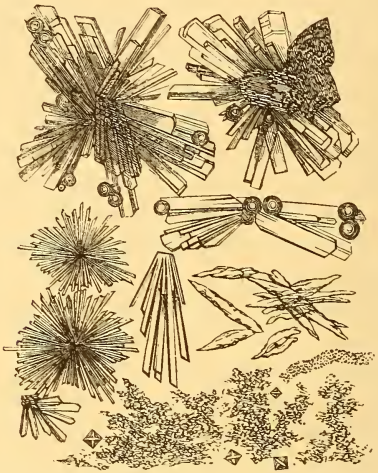
In the course of these changes, also, the acidity of the urine is diminished, and it may become neutral and even alkaline before the phenomena of the next stage to be described—the alkaline fermentation—set in.

2. After a still longer but variable period, which is shorter in warm weather and longer in cold, we have the so-called *alkaline fermentation*, which is a real fermentation. This, in which decomposing mucus is also thought by some to be the ferment, is ascribed by Tieghen* to the action of a little torula, structureless and without a cell-wall, which multiplies by budding, not at the surface, but within the urine or at the bottom of the vessel, where it with the deposited salts forms a white sediment. In this fermentation we have the urea converted into carbonate of ammonium,

* Neubauer and Vogel, Analyse des Harns, vi Auflage, 1872, pp. 110 and 130.

as already explained, by the addition of two equivalents of water.* As the result of this conversion, the urine is ren-

Fig. 16.



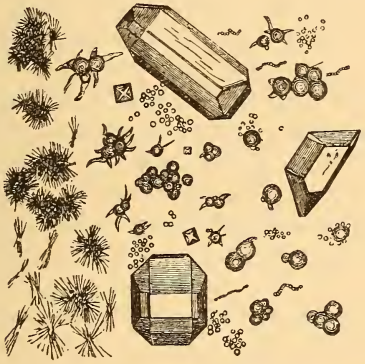
Prismatic crystals of sodium urate, spherules of ammonium urate, and amorphous urates, with octahedral crystals of oxalate of lime. (Ranke.)

dered highly alkaline, and a further change in the character of the sediment takes place. At the very beginning of the

* An explanation of the delay which sometimes occurs in the appearance of these phenomena is based on the recognition of the multiplication of these spores as the cause of the fermentation. If infusoria are simultaneously developed, the urea is more slowly converted, and if the surface of the urine happens to be covered with other plant vegetation (mildew), as is sometimes the case, the urine may remain acid for months in consequence of the interference with the access of oxygen, on the presence of which the spore is dependent for its growth and multiplication.

reaction, when the urine may still be neutral or even weakly alkaline, the uric acid crystals begin to dissolve and to change their form so as to become more or less unrecognizable, while on their fragments may often be seen to adhere prismatic crystals of urate of sodium and dark spheres of urate of ammonium (Fig. 17). As the reaction becomes alkaline, the uric acid altogether disappears, and the field becomes crowded with granules of amorphous phosphate of lime, beautiful triangular prisms, ("coffin-lid" shaped crys-

FIG. 17.



Spiculated spherules of ammonium urate along with triple (ammonio-magnesian) phosphate and octahedral crystals of the oxalate of lime. (Ranke.)

tals) and their modifications, of the triple phosphate of ammonium and magnesium, and opaque black balls of urate of ammonium often beset with spiculæ (Fig. 17); the spores referred to are also often present, while millions of bacteria vibrate slowly along, or form granular aggregations about a fragment of organic matter, and an occasional infu-

sorium darts across the field of view with magnified celerity. Commonly, however, the intermediate stage is lost sight of, and the stage just described is the only one seen in the alkaline fermentation. Such urine has an ammoniacal and putrescent odor, is cloudy from the suspended phosphate of lime and bacteria, and exhibits to the naked eye an abundant white deposit.

Either of the above set of changes may take place within the body, that is, in the pelvis of the kidney or in the bladder, and as such form pathological conditions which are constantly met with in practice, the first in the condition of uric acid gravel or calculus, with its incident suffering, and the second in the phenomena of irritation and inflammation, more particularly of the bladder, due to obstruction by stone, stricture, or malignant disease. It also seems to be a matter of modern observation that the germs of the fungi above alluded to, which appear to have a very close relation to the phenomena described, either as cause or effect, may be introduced from without by the use of imperfectly cleansed catheters, sounds, or similar instruments.

With this preliminary knowledge of the *rationale* of the causation of a large proportion of urinary deposits, we are ready to take up their detailed consideration, previous to which, however, allusion must be made to—

Extraneous Substances found in Urine.

These are very various, and include indeed all substances which are liable to get into vessels containing urine. The most common among these are fibres of cotton and linen, hair of blankets, worsted, wool, human hair, cats' hair, splinters of wood, oil-globules, starch-corpuscles, tea-leaves.

bread-crumbs, etc. With the microscopical appearances of all these the student should familiarize himself before he begins the examination of urinary sediments.

Scratches and **marks** in the glass slides may also confuse, if not mislead, the beginner, and, if they become filled with coloring matters are more likely to do so. Such error was, for a long time, occasioned by the pigmented markings often found in glass slides, which were so long and so often described by observers as *pigment flakes*. They are little depressions or scratches in the glass which have become filled with oxide of iron used in the polishing of the glass. Their true character was first pointed out by the late Dr. J. G. Richardson, of this city.

Classification of Urinary Deposits.

Efforts have been made to classify sediments on different bases, that is, on the ground of their external naked-eye characters as to bulk, color, weight, etc.; again with regard to their nature and origin, whether organized or unorganized, crystalline or amorphous; and finally as to the reaction of the urine in which they are found.

The simplest division is into *unorganized* and *organized*. A further division of these groups into crystalline and amorphous seems to separate groups which are naturally associated, and is therefore omitted.

Unorganized.

I. Uric acid (crystalline).

II. Uric acid compounds.
- *a*. Acid sodium urate (amorphous, occasionally crystalline).
- *b*. Acid potassium urate (amorphous).
- *c*. Acid calcium urate (amorphous).
- *d*. Acid ammonium urate (crystalline).

III. Oxalate of lime (crystalline).

IV. Earthy phosphates.
- *a*. Ammonio-magnesian phosphate (crystalline).
- *b*. Calcium phosphate (amorphous and crystalline).

V. Carbonate of lime (crystalline).

VI. Leucin and tyrosin (crystalline).

VII. Cystin (crystalline).

Organized.

I. Mucus and pus.
II. Epithelium.
III. Blood.
IV. Casts.
V. Spermatozoids.
VI. Fungi and infusoria.
VII. Elements of morbid growths.
VIII. Entozoa.

Unorganized Sediments.

I. Uric Acid.

Occurrence.—Uric acid presents itself as a sediment of small bulk, sinking to the bottom, but sometimes also adhering to the side of the glass. The individual crystals are often large enough to be seen by the naked eye, and in their aggregation frequently form masses so large as to be characterized by the terms "sand," "gravel," "red-pepper grains." This latter term is based upon the *red* or *yellowish-red* coloration which uric acid crystals in urine exhibit.

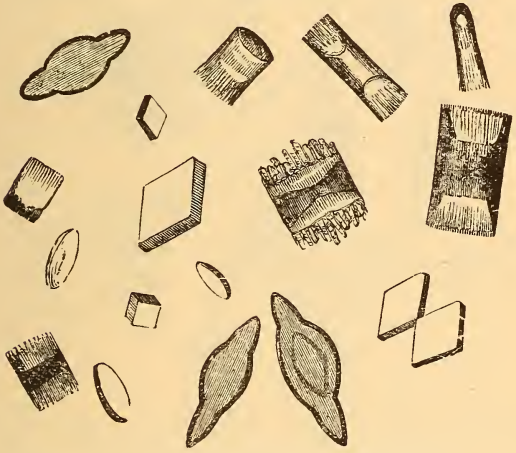
They are found perfect only in acid urine, often at the end of the so-called acid fermentation, in urine concentrated from any cause, and where there is a pathological increase in the production of uric acid due to imperfect oxidation or assimilation.

Recognition.—The typical shapes of a uric acid crystal may be said to be a *four-sided* rhomb and *six-sided* plate.

But it is comparatively seldom that the typical forms are

observed, the latter shape being somewhat rare, and the angles of the former being generally so rounded off that the crystals assume an ovoid or "whetstone" shape, of very different sizes, some being mere points with powers of 200 to 300 diameters, while others are large enough to be seen by the naked eye. Further shapes are those of sections of a barrel, envelope, spear, fan, of a comb with teeth on two sides, quadrilateral prisms with terminal planes, dumb-bells, and even other forms. What are commonly called

FIG. 18.

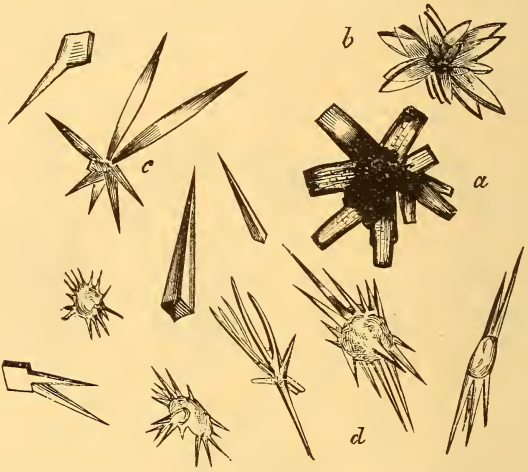


More usual forms of uric acid crystals. (After Harley.)

"dumb-bells" of uric acid may be rather compared to a tuft of hay constricted at its middle. These varied forms practice soon teaches one to recognize, even though they may deviate much from the typical shape. Uric acid

crystals, as mentioned, are *almost invariably* colored, and can generally thus be distinguished from other deposits. Dr. Beale* states that two or three instances have come under his notice in which they were not colored, and I have met such crystals. Uric acid crystals are met singly, but very commonly they are aggregated, forming beautiful

FIG. 19.



More unusual forms of uric acid crystals. (After Harley.)

rosettes and other shapes of aggregation of such size as to be easily visible to the naked eye,—as the "red-pepper grains" already alluded to,—and give pain in their transit through the ureter.

Fig. 18 exhibits the more usual varieties of uric acid, and Figs. 19 and 20 some of the rarer forms.

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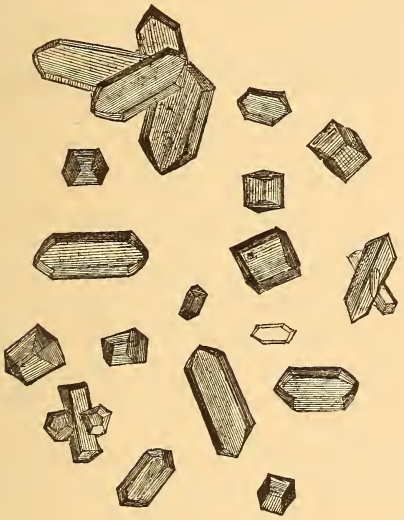
* Kidney Diseases and Urinary Deposits, Philadelphia, 1869, p. 371.

Tests for Uric Acid.—Whenever a crystalline deposit is of doubtful character and suspected to be uric acid, if the latter it will respond as follows:

1. Insoluble in cold or hot water, it will readily dissolve in the alkalies, soda, potash, or ammonia. If then the

FIG. 20.



Other unusual forms of uric acid, not unlike crystals of the triple phosphate of ammonium and magnesium. × 150.

alkaline solution be treated with an excess of acetic acid, in a few hours typical whetstone shaped forms will crystallize out.

2. Or the sediment may be placed on a glass slide, and treated with the murexid test, as described on page 160.

The dumb-bell crystals of uric acid, occasionally met with, may be distinguished from the dumb-bell crystals of the oxalate of lime by the characteristic shape already referred to, by their larger size, their darker color, and their solubility in alkalies.

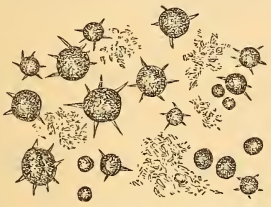
II. Uric Acid Compounds.

(*a*) *Sodium urate*, mainly amorphous, is sometimes crystalline. It always forms a part, and, according to Bence Jones, a predominant part, in the pulverulent, heavy, variously tinted, and generally bulky deposit of the mixed urates known as "brickdust" or "lateritious" sediment. The degree of coloration of this sediment depends upon that of the coloration of the urine whence it falls. From pale urine of low specific gravity, 1010 to 1014, an almost *white* sediment separates, falling very slowly, and producing, therefore, an opaque cloudy appearance in suspension, but readily disappearing on the application of heat; from urine of an amber color, and specific gravity of about 1018, the urates deposited are fawn-colored; and from high-colored urine of higher specific gravity, we have the true red "brickdust" sediment. The sediment is found in acid urine, or urine in which the acid fermentation has only commenced, and has not been operating so long as completely to remove the base and cause the crystalline uric acid to be deposited. It is found also in urine concentrated from any cause, or where it has cooled down considerably below 37° C. (98½° F.), or where there is defective oxidation or assimilation, as in fevers.

Recognition.—By far most frequently do we find sodium urate in fine amorphous granules, by their shape in no wise distinguishable from other fine granular matters, requiring,

therefore, the chemical tests for their discrimination. The adhesion of these fine granules to partially coagulated shreds of mucus sometimes give rise to an appearance resembling finely granular casts (Fig. 22), which is readily detected by the experienced, but which may mislead the beginner. The careful application of heat, or the addition of a drop of acetic acid, will promptly dissipate the illusion. These granules of sodium urate also assume a larger size, and become little spherules, sometimes provided with spicules, which are considered by some (G. Bird, Beale) to be spicules

FIG. 21.

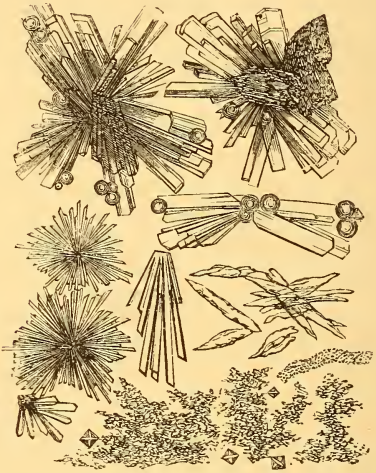


Spherules and spiculated spherules of urate of ammonium (sodium?); amorphous granular urates.

of uric acid. (See Fig. 21, from Beale, *Kidney Diseases.*) Other spherules are provided with projecting and curved processes, and are believed by Hassall (second edition, page 75) and Thudichum (second edition, page 81) to be composed of sodium urate throughout. That the *spines* were also urate of sodium, Thudichum considered evidenced by their solubility in water. A modified form of the latter are probably the irregularly star-shaped crystals in Dr. Beale's Fig. 110, from the urine of a patient suffering with peritonitis. But all of these forms of spherules with straight and incurved processes (thorn-apple shapes) are put down

by the German observers (Neubauer and Vogel, Hoffmann and Ultzmann) as crystalline forms of urate of *ammonium*, in which I am inclined to concur, at least with regard to those which are found at the stage of reaction intermediate between the acid and alkaline fermentations, or, perhaps, rather at the beginning of the latter, when ammonia makes

FIG. 22.



Prismatic crystals of acid sodium urate, spherules of ammonium urate, and amorphous urates with octahedral crystals of oxalate of lime. (Ranke.)

its appearance, and is accompanied by the ammoniomagnesian phosphate. But any spherules which occur early in the acid reaction, or before it is possible for any ammonia to be present, are probably *sodium urate*.

The sodium urate is also rarely found in *dumb-bells*, which are also striated and broad at the extremities like those of uric acid, but less disposed than the latter to break

up at the extremities into individual acicles (Atlas of Hoffmann and Ultzmann, Taf. IX). One-half of one of these dumb-bells, viewed from above, would be fan-shaped.

Under the same circumstances, at the end of the acid and at the beginning of the alkaline fermentation, do we also have the true prismatic *crystals* of acid sodium urate arranged in star-like masses (Fig. 22).

(*b*) *Acid potassium urate* is also amorphous, very soluble, and occurs under the same circumstances as sodium urate, as a constituent of the mixed urates.

(*c*) *Acid calcium urate* occurs very seldom, and in small quantity, as a white amorphous powder, along with the mixed urates. It is with difficulty soluble in water, and known to have lime for its base, by leaving a residue of calcium carbonate after incineration.

(*d*) *Acid Ammonium Urate.*—This is found, along with amorphous earthy phosphates and crystals of the triple phosphates of ammonium and magnesium, in urine in which the alkaline fermentation has commenced. It is the only urate found in alkaline urine.

Recognition of Ammonium Urate.—It is crystalline, and presents itself in the shape of smooth and characteristic "thorn-apple" spherules (Figs. 21 and 22), which serve easily to distinguish it. The spherules are soluble in hot water, and dissolve in hydrochloric or other acid with the evolution of uric acid crystals. Liquor potassæ, added to them, evolves the odor of ammonia, and they give the murexid reaction with nitric acid and ammonia.

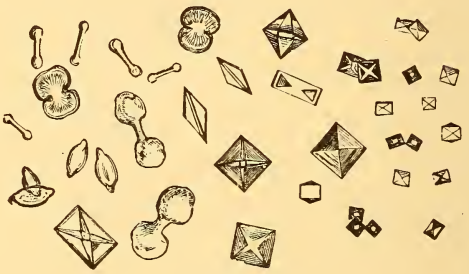
Tests for Acid Urates.—Though the acid urates are much less soluble than the neutral urates, in requiring 124 parts of boiling water and 1150 of cold, they readily dissolve on the application of heat to the slide or test-tube

containing them. They are dissolved also by the alkalies, liquor potassæ or sodæ. Treated with nitric, hydrochloric, or acetic acid (the diluted are better on account of their slower action), they dissolve, with the subsequent crystallization of uric acid. They also respond to the murexid test.

III. Oxalate of Lime.

Occurrence.—The oxalate of lime crystals are most frequently met in acid urine, often therefore alongside of crystals of uric acid, but they may also be met in alkaline

Fig. 23.



(After Harley.)

urine, along with crystals of the triple phosphate. They are particularly abundant in the urine after a meal of rhubarb plant, after the use of tomatoes and other vegetables containing oxalic acid. There are no means by which the presence of oxalate of lime may be foretold before a microscopic examination of the urine is made. The first edition of this book contained the following: "It never forms a deposit appreciable to the naked eye, and most commonly the crystals do not descend to the bottom of the glass, but are caught as it were by the flocculi of mucus which float *towards* the

bottom, rather than occupy it." Later and repeated observations have convinced me that in many instances the whole of this cloud-like mass, so much resembling mucus, is made up of oxalate of lime.

Recognition.—Two forms of calcium oxalate crystals are met, the *octahedra* and the *dumb-bell* crystals. The appearance of the former is somewhat different according as they are seen in the longer diameter or in the shorter. They may be said to be made up of two four-sided pyramids, placed base to base, and when viewed in the longer diameter may readily be detected as such by the microscope. When seen in the opposite direction, their characteristic appearance is that of a square, crossed obliquely by two bright lines, and if the crystal be very small, it will appear as a square with a bright spot in the centre—a characteristic appearance by which one may soon learn to detect them, even when they are very small. They are often seen in aggregations of three, four, or more, closely adherent, and forming as it were microscopic calculi.

The *dumb-bells*, very much more rarely met with, are highly characteristic; and although we have spoken of dumb-bells of uric acid and of ammonium urate, neither of the latter presents the typical dumb-bell appearance like those of the oxalate of lime. In addition to these are found allied forms, circular and oval shapes, with darker or brighter centres, and some with partial concavities at the sides, as though passing over into dumb-bells. Dumb-bells are also met aggregated, forming microscopic calculi, which go far to explain the incipient formation of calculi.

Chemical Characters.—The form of crystals of oxalate of lime is so characteristic that there is seldom occasion to make use of the chemical tests to determine them. The only

crystals which at all resemble them are certain forms of the triple phosphate. These are small crystals, modifications of the typical triangular prism, with its bevelled ends, in which the body of the prism is exceedingly short, as if it were almost left out, so that the two inclined triangular ends closely approach each other, and form a crystal like that of the octahedron of oxalate of lime. Their nature may, however, be suspected by the shape of the larger crystals around them, for they never occur alone. Moreover, they are promptly dissolved by acetic acid, while the oxalate of lime is totally insoluble in this acid. The octahedra are highly insoluble in water, in alkalies, and in the vegetable acids, including acetic, but are soluble in the mineral acids. The dumb-bells, after the prolonged action of acetic acid, yield their crystalline matter, leaving a frame-work, which maintains the original shape of the crystal. This in fact explains, perhaps, the shape of the crystal. It has been shown by Mr. Rainey and others that the presence of organic matter, as mucus, interferes with the crystallization in the regular manner. The dumb-bells of oxalate of lime can readily be distinguished from the dumb-bells of uric acid or urates by the solubility of the latter in alkalies.

The acid phosphate of sodium, according to Neubauer,* possesses a power of solution over the oxalate of lime, often holding it in solution, and he gives a method by which the latter may be obtained from solution in the urine by its agency, as follows: 400 c.c. to 600 c.c. (13.3 to 20 f℥) of the urine to be tested are treated with solution of chloride of calcium, supersaturated with ammonia, and the pre-

* Neubauer and Vogel *op. citat.*, p. 174.

cipitate dissolved in acetic acid. After twenty-four hours, the precipitate then occurring, which nearly always contains uric acid, is placed on a filter, washed with water, and a few drops of hydrochloric acid poured upon it. The latter dissolves out the oxalate of lime present, and leaves the uric acid on the filter. The filtrate is then diluted in a test-tube with 15 c.c. (2.83 f℥) of water, and overlaid most carefully, by means of a pipette, with very dilute ammonia in sufficient quantity. At rest, the two fluids gradually mingle, and after twenty-four hours the oxalate of lime present will have collected at the bottom, and octahedra of great beauty may be studied with the microscope.

Neubauer says he has many times, in this manner, obtained considerable quantities of oxalate of lime, where there was previously no deposit whatever. He has, however, in other instances, with normal urine obtained negative results, so that he is unable to decide whether the oxalate of lime should be considered a normal or abnormal constituent of urine.

Sources of Oxalate of Lime in the Urine.—There is no doubt that *oxalic acid* is, at times at least, secreted by the kidneys, and meeting immediately the lime salts, for which it has a strong affinity, forms the crystals we are considering; for both octahedra and dumb-bells are not infrequently found in the uriniferous tubules of the kidney, and even in tube-casts. Schunck has attempted to show that the oxalate of lime is formed during the decomposition of urine from the oxalate of ammonium, but Neubauer says the oxalate of ammonium is converted into carbonate of ammonium. Others, as Owen Rees, Aldrige of Dublin, Wöhler, and Frerichs, allege that oxalate of lime is

derived from a decomposition of uric acid and urates. Their experiments would seem to show this, and it is undoubtedly the case that deposits of oxalate often make their appearance in urine some time after it has been passed. Two sources must, therefore, be admitted, one within the organism and one without.

Clinical Significance.—There is no disease with which the oxalate of lime is particularly associated, nor can deposits of it be considered indicative of derangement. Abundant deposits of oxalate of lime are found in the urine of persons who are typically healthy. On the other hand, it is apt to occur where there is mal-assimilation, and hence dyspeptics are often found having oxalates in their urine, as a result rather than a cause of the affection from which they suffer.

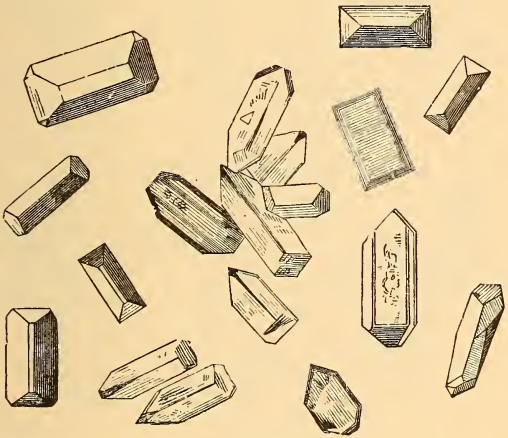
When there are symptoms of renal calculus descending from the pelvis of the kidney, and oxalates are found in the urine, especially if they form the aggregation referred to, the latter may afford explanation of the nature of the stone. Unfortunately, too often there is no sediment whatever attending the descent of a calculus, and we must, therefore, determine its nature without such aid, or remain in ignorance. A careful examination should, however, always be made of the urine in nephritic colic, as valuable information is at times at least furnished by it, especially in the uric acid lithiasis, where uric acid sediment is often found.

IV. Earthy Phosphates.

Occurrence.—These deposits are found only in feebly acid or alkaline urine, and are the more abundant the more advanced is the stage of alkaline fermentation. They ap-

pear to the naked eye as bulky opaque white deposits, unless they are accompanied by blood, which then more or less tinges them. The urine itself is apt to be turbid from the presence of amorphous phosphate of lime in suspension, to have an ammoniacal and sometimes a fetid odor, though not necessarily. They are especially abundant in the urine of all irritative affections of the bladder, and

FIG. 24.



(After Harley.)

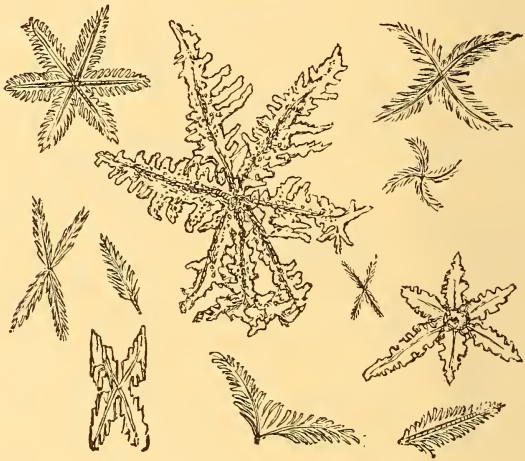
often attend diseases of the spinal cord, because of paralysis of the bladder and consequent retention of urine. The earthy phosphates are the *triple phosphate* or ammonio-magnesian phosphate and the *phosphate of lime.*

(*a*) The **ammonio-magnesian phosphate** ($MgNH_4PO_46H_2O$), or triple phosphate, is a crystalline deposit, of

which the typical form is a triangular prism (Fig. 24) with bevelled ends, very characteristic and easily recognized.

In addition to this, there is an infinite variety of modifications, with one or more corners removed, the body of the crystals variously shortened, etc. Among these forms are the small crystals already referred to as being possibly

FIG. 25.



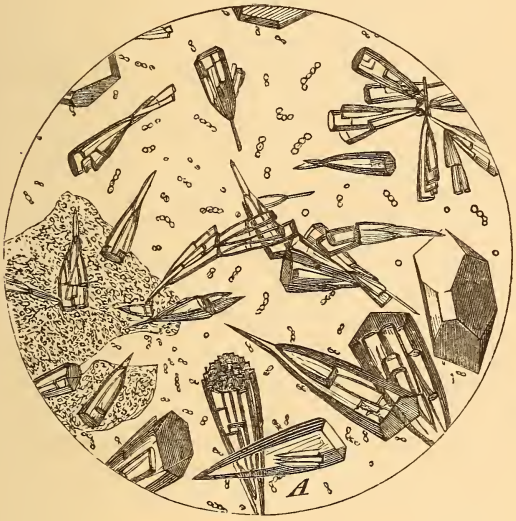
(After Harley.)

mistaken for the oxalate of lime. There are also sometimes found beautiful star-shaped feathery (Fig. 25) crystals of triple phosphate, which gradually undergo conversion into the prisms, and between these two there are many intermediate forms.

(*b*) **Phosphate of Lime** (amorphous $Ca_3(PO_4)_2$, crystalline $CaHPO_4$).—Phosphate of lime is most frequently

found amorphous under the same circumstances under which the triple phosphate occurs. It is, however, frequently deposited from normal urine by which it is held in solution during the acid reaction by the acid phosphate of sodium, or carbonic acid, or by both. At any rate let the acid re-

Fig. 26.



Crystalline and amorphous phosphate of lime.

action be wanting, as it is three or four hours after a meal, and a copious deposit of calcium phosphate often takes place, and is increased by boiling. In other instances, a urine may be acid in its reaction, and the boiling, apparently by driving off the carbonic acid, will cause the phosphates to go down. These deposits have more than once

been spoken of as possible sources of error in testing for albumin, but they promptly disappear on the addition of acids. The color of the phosphate of lime alone is not snow-white, as is that of the triple phosphate, but rather yellowish.

Not unfrequently we meet in urinary deposits *crystalline phosphate of lime* (Fig. 26), which occurs sometimes alone and sometimes along with the triple phosphate. It is also met in urine of a weak acid reaction, but strongly disposed to take on the alkaline fermentation. The occurrence of crystalline phosphate of lime seems peculiar to certain individuals, and Hoffmann and Ultzmann have met persons perfectly healthy who, in the summer months, have almost daily deposits of crystalline phosphate of lime. It is frequently associated with octahedra of the oxalate of lime.

Recognition.—The isolated crystals of phosphate of lime may be said to be *wedge-shaped* or even *conical*, from which form there are, however, variations. But their characteristic feature is in their arrangement, which is that of a circular rosette, in which the apices of the numerous crystals forming it all point to the centre. Phosphate of lime is also found in the shape of spherules or even *dumb-bells*. The latter are said by Dr. Beale (*Kidney Diseases and Urinary Deposits*, p. 357) to be deposited in decomposing mucus, not only from the urinary tract, but from other surfaces, as the gall-bladder. Dr. Beale figures such dumb-bells in his Plate XXI, Figs. 116 and 118.

Chemical Characters.—All of the earthy phosphates are dissolved by acids, but are precipitated by alkalies and heat, whereas the uric acid salts are dissolved by both these agencies. The small triple phosphate crystals, which resemble those of oxalate of lime, dissolve quickly in acetic acid,

while the octahedra are untouched by it. Uric acid itself could scarcely ever be confounded with phosphates, occurring, as it does, in urine of different reaction; but if it were necessary to discriminate them, the former are dissolved by alkalies, the latter not. Moreover, the murexid test will not respond to phosphates, but will to uric acid.

V. Carbonate of Lime

is a very rare deposit in human urine, but is found abundantly in horse's urine. When present, it occurs in small spheres, and is detected by its effervescence with acetic acid.

VI. Leucin and Tyrosin.

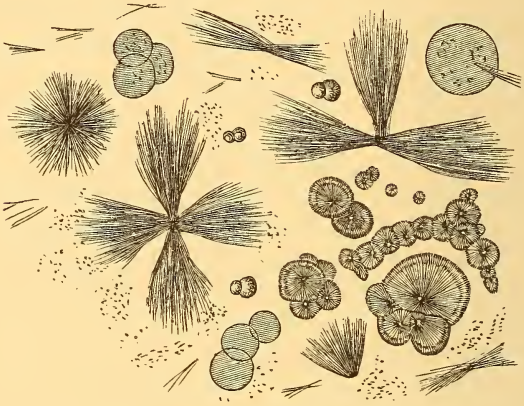
Occurrence.—These crystalline deposits are only found in urine which is loaded with biliary coloring matters, since they attend only grave destructive diseases of the liver, especially acute yellow atrophy and phosphorus poisoning.

Recognition.—If suspected in urine presenting the above characters, it may be slightly evaporated, when the crystals will usually be deposited if leucin and tyrosin are present.

Leucin presents itself in the shape of more or less yellow-tinged, highly refracting spheres, which may at first sight be taken for *oil-drops*. A little study will show them refracting light not quite so strongly, *i. e.*, not possessing quite so wide a dark border; and by suitable illumination many of them will be found marked with radiating and concentric striæ. The spherules further exhibit a peculiar disposition to aggregate, appearing partially to merge where two edges come together.

Chemical Characters.—Leucin spheres, unlike oil-globules, are insoluble in ether, and further are soluble in caustic alkalies, but not in cold mineral acids. Spherules of sodium urate resemble somewhat leucin spheres. The former are, however, soluble on being warmed, and may also be recognized sometimes by the development of spines on their surface.

FIG. 27.



Leucin spheres and tyrosin needles.

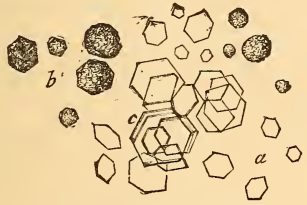
Tyrosin is found in the shape of very fine needles arranged in tufts or "*sheaf*"-like collections, often crossing each other and intersecting at their constricted central portions (Fig. 27).

Chemical Characters.—Tyrosin may be recognized by Hoffmann's test. A suspected deposit is boiled in an excess of water. To the boiling fluid a few drops of a solution of mercuric nitrate are added, and there arises a red precipitate, while the supernatant fluid is colored red to purple-red.

VII. Cystin ($C_3H_7NSO_2$).

Occurrence and Recognition.— Cystin is a rare urinary sediment. Crystalline, forming a whitish or dirty yellowish-gray deposit, which on microscopic examination is found to be made up of regular six-sided tablets of different sizes, often so arranged that one of smaller size is superimposed on one of larger, and this upon a still larger, and so on; but it also occurs in irregular masses (Fig. 28). It is usually met in a pale urine, both acid and alkaline, developing in decomposition the odor of sulphuretted hy-

Fig. 28.



Cystin. (After Harley.)

drogen, as well as that of ammonia, the former doubtless derived from the sulphur contained in the cystin. It occurs as a separate urinary deposit as well as accompanying cystin calculus, which seems sometimes to be hereditary.

Chemical Characters.—It is soluble in ammonia, and, upon spontaneous evaporation of the ammoniacal solution, *the six-sided crystals reappear*, showing that it is simply dissolved in the ammonia, and not in chemical combination with it. Now if the six-sided crystals of uric acid, which so closely resemble it, and which often accompany it, are dissolved in ammonia, and the solution allowed to evapo-

rate, there would be formed ammonium urate, and, on evaporation of the solution, this ammonium urate would remain as an *amorphous residue*. Cystin is also insoluble in boiling water, in strong acetic and very weak hydrochloric acids; but it is readily soluble in oxalic and strong mineral acids. It is soluble in potash and insoluble in solution of carbonate of ammonium, and therefore may be precipitated from an acid urine by the alkaline fermentation; under these circumstances it would be accompanied by amorphous phosphate of lime and crystalline phosphate of ammonium and magnesium, with neither of which is it likely to be confounded. In a mixed deposit containing six-sided crystals, the lime and triple phosphate may be dissolved out with acetic acid, while the plates of cystin will remain. They may then be treated with ammonia, as above, to distinguish them from uric acid.

Cystin contains 26 per cent. of sulphur.

Organized Sediments.

I. Mucus and Pus.

Mucus must be present in considerable amount to be recognized by its own properties, since it is so transparent and similar to urine in its refractive index. It is visible partly through the accidental morphological constituents which it more or less constantly holds in suspension. These are the so-called mucus-corpuscles and epithelium from all parts of the genito-urinary tract, as well as crystals of the oxalate of lime, granules of sodium urate, and even crystals of uric acid. In strictly normal urine the first two would alone be present, and in very minute quantity. Mucus, when present in normal amount, appears as a *delicate* cloud,

often barely visible, floating *towards* the bottom rather than at the bottom of the vessel.

By the action of *acetic* acid, the *mucin*, an element of mucus which is comparable to albumin, though not coagulable by heat, is precipitated in the shape of delicate fibrillated bands, which are sometimes tortuous, and again appear as delicate threads, known as mucin threads. If a little iodine and iodide of potassium be added to the acetic acid, they are made even more distinct. Tartaric acid and very dilute solutions of the mineral acids have the same effect, while an excess of the same will redissolve the precipitate; so, too, the mineral acids will dissolve the coagulum of acetic acid, while an excess of the latter will not dissolve it. These coagula may sometimes be found in urine to which no acids have been added, being probably produced by the action of the acids developed in the acid fermentation. Under these circumstances they are particularly apt to be studded with granular urates, which may cause them to be mistaken for granular tube-casts; but they are generally very much narrower than the latter, and the addition of a little warmth, hydrochloric acid, or alkali will quickly dissolve the granules (see Fig. 22).

As the result of irritation of any part of the genito-urinary tract, mucus is increased in quantity, when it assumes a thicker, more ropy character, and becomes more or less opaque; but even here the opacity is largely due to the increased proportion of cellular elements. Under these circumstances, the opaque clouds of mucus are often enormously increased, and with them the adherent epithelial cells from the seat of irritation. When thus in excess, mucus is apt to pervade more or less the entire mass of the urine rather than sink to the bottom, giving the entire fluid,

therefore, a glairy character. Mucus, however, seldom becomes very abundant without being attended by pus, as the causes producing them are but differences of degree. So long, however, as urine containing mucus is without *albumin*, so long may pus be said to be absent, as *mucus itself contains no albumin, while pus does.*

The Mucus- and Pus-corpuscles.—The *mucus-corpuscle*, as it appears in urine, is a small, granular, spherical or nearly spherical cell, rather larger than a blood-corpuscle, that is, .008 to .010 millimeter ($\frac{1}{3000}$ to $\frac{1}{2500}$ of an inch) in diameter, containing one or more nuclei. In a healthy condition of mucous membrane, a mucus-corpuscle, however it originates, is nothing more nor less than a young epithelial cell which has reached the surface before it has attained the characters of such cell in its development. As such, therefore, we must not too closely restrict its size, for who shall say where the mucus-corpuscle terminates and where the epithelial cell begins? As such a young cell, without morbid impression, simply arrested in its normal development, a single nucleus is more common than it is in the *pus*-corpuscle, of which the multiple nucleus may be said to be more characteristic. But here the difference ceases. For the pus-corpuscle, when young (that is, not the subject of fatty degeneration), is a cell exhibiting the same characters, and may be defined in the same way. The fact being that when a cell exhibiting the above characters, with one or multiple nuclei, is found upon a non-suppurating surface, it is called a mucus-corpuscle, while the same cell on a suppurating surface would be called a pus-corpuscle. Thus, while the two are physiologically distinct, they are anatomically the same, the physiological difference being in this, that a pus-corpuscle is a cell too rapidly produced to be

allowed to develop into the normal tissue of the part, while the mucus-corpuscle is, as it were, only accidentally arrested in its development. The same resemblance which exists between these bodies exists between them and the white corpuscles of the blood, and to the whole class of cells to which the term *leucocyte* or white cell is conveniently applied.

The Action of Reagents.—The mono-nucleated mucus-corpuscle, which may be considered an older mucus-corpuscle, or young epithelial cell thrown off at a later period, usually exhibits its single nucleus distinctly, without the addition of a reagent; but the majority of leucocytes have not their nuclei visible until acted upon by certain

FIG. 29.

Mucus- and pus-corpuscles before and after the addition of acetic acid.

reagents, of which two acting similarly most interest us. These are water and dilute acetic acid.

1. **Action of Water.**—When water is added to the pus- or mucus-corpuscle, its first effect is to cause the latter to swell up, sometimes to twice the original size, next to become smooth, the granules gradually disappearing, while the nuclei come out with great distinctness. Finally, after some time the body of the cell becomes almost, and then quite invisible, while the nuclei remain some time longer. The circumstances under which the corpuscle exists in urine are not quite identical, because in it we have a solution of

organic and inorganic matters considerably denser than water, having a sp. gr. 1015 to 1025, and while the action is somewhat similar, it is very much slower; and if the specific gravity of the urine should be very high, exceeding that of the fluid in the cell, there might be no effect, or a contrary one, *i.e.*, a shrinkage of the cell from an exosmosis of its contents.

2. **Acetic Acid.**—The action of dilute (20 per cent.) acetic acid is identical with that of water, except that it is very much more rapid, and the stage of distinct nuclei is reached much sooner.

3. The **caustic alkalies** have a rapidly solvent effect upon these corpuscles, destroying their morphological identity, and converting them into a gelatinous adherent mass.

Characters of Urine containing Pus.—Urine, containing pus, deposits an opaque white sediment, which sinks rapidly to the bottom, so long as the reaction is acid and there is no mucus present. Such urine, when shaken up, becomes more or less opaque, according to the amount of pus which it contains. The opacity, as well as the deposit, often resembles that due to the pale granular urates, from which both are distinguished by the disappearance of the latter on the application of heat, while *purulent urine* deposits albumin under the same circumstances. To a less degree does urine containing pus resemble that containing amorphous phosphate of lime; but the latter is dissipated by acids, while acids also precipitate the albumin from pus, and the microscope reveals hundreds of the granular cells already described as pus-cells, in many of which the nuclei are already displayed in consequence of the action of water.

Donne's test for pus is based upon the reaction referred to between the alkalies and pus. It consists in the

addition of liquor potassæ to the suspected deposit, after the supernatant urine is poured off. If the deposit is pus, it is promptly converted into a viscid, gelatinous substance *resembling mucus*, which adheres to the bottom of the test-tube, often permitting its inversion without falling out, and which, when it is forced to flow, does so in a continuous mass, as the albumen runs out of a broken egg. If a portion of this glairy mass be examined under the microscope, the pus-corpuscles will be found to have been destroyed, or, rather, converted into the substance itself. If the action has not been very long, or the proportion of alkali to the pus is small, the nuclei of the corpuscles may still be found as black dots in the mass, or a certain proportion of the corpuscles may preserve their integrity.

Changes in Urine containing Pus.—On this same reaction is based a most important change which urine containing pus undergoes after the alkaline fermentation has set in. Through the agency of the carbonate of ammonium generated, precisely the same change is wrought, and the urine contains a deposit so firmly adhering to the bottom of the bottle that it is impossible to remove it with a pipette. It must be remembered that *this is not mucus*, although it so closely resembles it, and although microscopic examination may show the total absence of pus-corpuscles. These have been dissolved by the alkali. Care should be taken, therefore, to determine the reaction of the urine before a mucoid deposit is decided upon, and if it is alkaline, another of acid reaction should be obtained. The glairy product referred to will be found dotted with glistening points, which, on microscopic examination, prove to be crystals of triple phosphate, while the super-

natant fluid will be found to contain albumin, which is wanting in deposits of pure mucus.

Frequently, in diseases of the bladder, these changes take place within the organ, forming a gelatinous mass, which plugs up the urethra and makes it almost impossible to evacuate the bladder, thus greatly increasing the suffering of the patient. In such cases the only remedy is to wash out the bladder with weak acid solutions, and having cleansed it, keep it so by their daily use. Even when acid at the time of being passed, these urines become rapidly alkaline afterward.

Sources of Pus in the Urine.—Pus in the urine may come from any part of the genito-urinary tract. When descending from the *pelvis* of the kidney, as it often does, where there is impacted calculus, it is less apt to be mingled with mucus, the urine retains its normal reaction, and the pus is, therefore, readily miscible with the urine, and as promptly deposited from it. When coming from the *bladder*, if the urine is not already alkaline, it is apt to become so very quickly, and we have then the phenomena described as incident to the alkaline fermentation, taking place soon after the urine is passed, if not in the bladder itself.

In diseases of the *prostate* are apt to be found long plugs of mucus, which, appearing to the naked eye like fine threads, upon microscopic examination are found made up of aggregated pus-corpuscles, in which are sometimes found the larger round, or nearly round, nucleated cells peculiar to this seat. Similar plugs are found in the pus from gonorrhœa, and it is said also that in this affection the mucus-corpuscles are distinguished from those derived from the bladder by their larger size, their "glass-like clearness" and diminished granulation. If there be no gonorrhœa,

these plugs or threads point almost pathognomonically to inflammation or irritation of the prostate.

In females, pus is apt to obtain in the urine from leucorrhœa or other purulent discharge from the vagina. This should not be forgotten.

II. Epithelium.

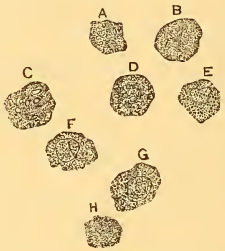
Epithelium from all parts of the genito-urinary tract is found in the urine, but it is not very often that we are enabled to locate its site beyond the bladder and vagina, partly because of the comparatively slight differences in the epithelium from different locations, and partly because maceration in the urine renders such feeble distinctive points even less marked.

Three varieties of epithelium may, however, be distinguished in urine with tolerable ease: 1st, round cells; 2d, cylindrical or conical and spindle cells; and 3d, squamous cells.

(*a*) **Round epithelial cells** (Fig. 30, and *a*, Fig. 31) arise from the uriniferous tubules, particularly in their convoluted portion, from the deeper layers of the mucous membrane of the pelvis of the kidney, from the bladder, and from the male urethra. Some of these cells, originally somewhat flattened by pressure, swell up in the urine and become nearly round (Fig. 30). They are distinguished from pus- and mucus-corpuscles by their larger size and their *single* nucleus, which is distinct without the use of reagents, while the *multiple* nucleus of the pus-cell requires the use of acetic acid to exhibit it. There is no way of distinguishing the source of these cells more precisely than as stated above, except that if the urine be albuminous,

and there is evidence of renal disease, it may be right to infer them to come from the tubules of the kidney, or from the pelvis if there are symptoms of impacted calculus; otherwise from the urethra, the prostate, Cowper's or Littre's glands, but cells from the latter are rare. If the plugs already referred to, made up of pus-cells with a few larger, nearly round, and distinctly mono-nucleated cells united by mucus, are present, we may infer the round cells to be

FIG. 30.



Round epithelial cells from the convoluted tubules of the kidney, found in urine from a case of acute nephritis. × 420. The cells A, E and H are slightly more granular than in health, and C contains a few oil-drops.

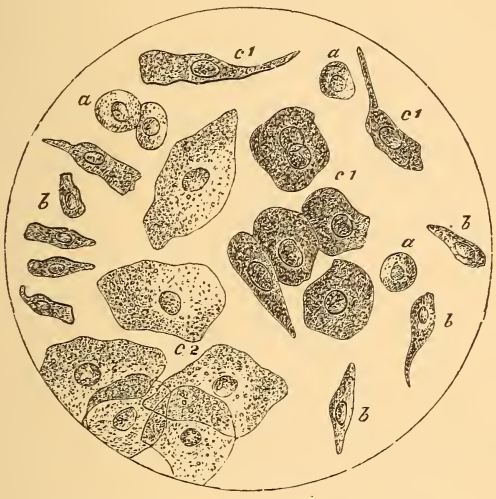
from the epithelium of the prostate. The round cells from the bladder are considerably larger than those from other sources—twice the diameter of a pus-cell.

(*b*) **Columnar or conical and spindle cells** (*b*, Fig. 31) are derived, the *first* from the superficial layers of the pelves of the kidneys, from the ureters and the urethra; the *latter* from the ureters and urethra.

(*c*) The **flat epithelial cells** (c^2, Fig. 31) arise from the bladder or the vagina. These are flat, but often thicker at the middle, contain a single nucleus, are irregularly poly-

gonal in outline, and often folded on themselves either completely or partially. The epithelial cells of the bladder (c^1) are not generally as large as those of the vagina (c^2), nor so flat; they are less apt to occur in layers or flakes,

Fig. 31.



a, Round epithelium from bladder.
b, Columnar epithelium from ureter and urethra.
c^1, Columnar and squamous epithelium from deeper layers of epithelium of bladder.
c^2, Squamous epithelium from superficial layers of epithelium of vagina.

although also found thus. Frequently it is not safe to attempt to distinguish between the two.

In acid urine these cells remain a considerable length of time, but in alkaline urine they are gradually destroyed, becoming at first swollen and more transparent.

III. Blood-corpuscles.

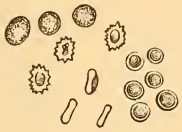
These get into the urine from the tubules and pelves of the kidneys, the bladder, the prostate, and from the uterus and vagina in their various physiological and pathological hæmorrhages. They may be so abundant as to be easily distinguished in mass by the naked eye, or they may require the microscope for their detection. Urine containing blood in large amount is impressed with the red color of the latter, but containing the moderate amount most frequently encountered in urine, it obtains a color depending on its reaction. If the urine is acid, it assumes a peculiar blackish-brown color which has long been described as "smoke-hued," and which is so characteristic as to enable one who is at all experienced to decide at once as to the presence of blood. If, on the other hand, the urine is alkaline in reaction, it assumes the bright-red color of blood. *Urine containing blood in any quantity appreciable to the naked eye is albuminous.*

If blood-corpuscles are present in numbers sufficient to produce an appreciable deposit, they form a brownish-red pulverulent mass at the bottom of the vial, if they come from the kidneys or ureters. They are more apt to be found in coagula if they come from the bladder or urethra, though this latter is not necessarily the case. On the other hand, moulds of clotted blood are sometimes discharged from the ureters with all the agonies of nephritic colic.

Recognition of Blood-corpuscles.—Blood-corpuscles are recognized under the microscope by the optical properties due to their biconcave centres. This is the *reversal of light and shadow* which they undergo in focussing, the centre and periphery alternating in bright-

ness or shadow as the object-glass is approximated to the slide or removed from it. This, in connection with their evident biconcavity when seen on edge, and their yellowish color, will always serve to distinguish them, although the effects of long-continued maceration tend to interfere in different degrees with the distinctness of all of these features. If the urine is a dilute one, the corpuscles will swell up, become biconvex instead of biconcave, finally spherical, and the reversal of light and shadow no longer occurs, while the coloring matter is more or less dissolved out. Ultimately the corpuscle altogether disappears. If, on the other hand, the urine is highly concen-

FIG. 32.



Blood-disks.

trated, the concavity becomes more marked and distinctive, while the corpuscle itself shrinks and becomes smaller, and soon acquires the crenated or horse-chestnut shape (Fig. 32).

In an acid urine the blood-corpuscles maintain themselves for a long time, but in an ammoniacal urine they are soon dissolved, being soluble in alkalies. The hæmatocrystalline and hæmatin are then dissolved in the urine, and may be tested for as already directed.

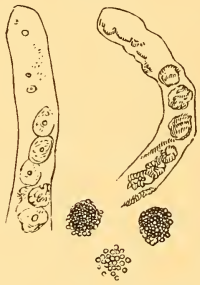
IV. TUBE-CASTS.

Tube-casts, "cylinders," as they are sometimes called, are moulds of the uriniferous tubules, produced by admis-

sion into the latter, by capillary rupture or otherwise, of a coagulable constituent of the blood, which there solidifies, and in this act entangles whatever it may have surrounded in its liquid state; subsequently it contracts and slips out of the tubule into the pelvis of the kidney, whence it is carried to the bladder and voided with the urine.

It should be added, however, that at least two other views as to the mode of formation of casts are entertained, according to one of which the cast is a result of the disintegration

FIG. 33.



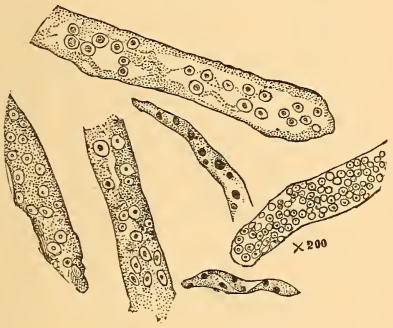
Epithelial casts and compound granule-cells.

and fusion of the epithelial lining of the tubules; and according to another, of a secretion from these same cells. That casts are sometimes formed according to the first, at least, of these two methods is not unlikely.

The mechanism of the production of the different varieties of casts, on the supposition of an albuminoid exudation from the blood is very simple. Thus, suppose a tubule to be filled with detached and loosely attached epithelium at the time the fibrin is poured into it. These elements are entangled, and, as the cast contracts, are car-

ried out in the shape of an "*epithelial*" cast (Fig. 33). If the tubule should happen to have contained blood, the cast entangling it is called a "*blood-cast*" (Fig. 34). Casts containing even a few blood-corpuscles are also called blood-casts. The basis substance of blood-casts is most probably the fibrin of the blood. If the epithelium be firmly attached to the basement membrane of the tube, and

FIG. 34.

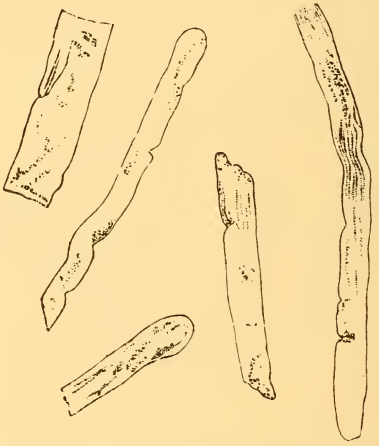


Blood-casts. (After Whittaker.)

remain behind when the cast passes out, or if the tube be entirely bereft of epithelium, then is the cast a "*hyaline*" (Fig. 35) or structureless cast. In the former instance the cast is of *smaller* diameter, and in the latter of *larger*, the diameter in the latter being that of the former plus twice the thickness of an epithelial cell. Fig. 36, *a*, from Rindfleisch, explains this sufficiently. From causes like these, as well as a subsequent contraction of the cast itself, the diameter of casts may vary considerably, ranging commonly from .01 to .05 mm. ($\frac{1}{2500}$ to $\frac{1}{500}$ in.). A cast is seldom completely hyaline, generally containing a few granules and

one or two glistening oil-drops, but it is still called *hyaline*. Completely hyaline casts do, however, occur. A variety of hyaline cast, more solid in appearance, and resembling molten wax, is spoken of as a "*waxy cast*" (Fig. 37). Some hyaline casts are so delicate as to be overlooked unless the

FIG. 35.



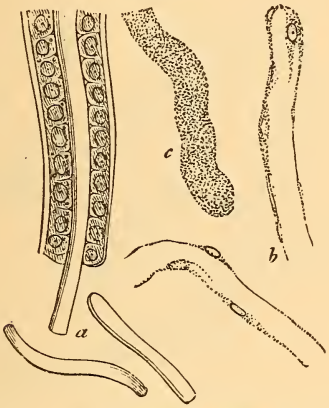
Hyaline casts. × 210.

light from the mirror illuminating the field of view be modified by shading with the hand or by manipulation of the mirror itself. If a cast contains granular matter, which is generally the granular débris of the degenerated epithelial lining of the tubule or of blood-corpuscles, it is called a "*granular*" cast, and *highly* granular (Fig. 36, *c*), *moderately* granular, *slightly* or *delicately* granular, according to the amount of granular matter present. When the material of granular casts is derived from broken-down blood-

corpuscles, the casts appear yellow or yellowish-red. Finally, if a cast is loaded with oil-drops, either free or contained in epithelial cells, it is called an "*oil-cast* or *fatty cast*" (Fig. 38).

Casts of smaller diameter are sometimes found within those of larger, the material of the latter having been poured out around that of the former after it has undergone some contraction. This occurs usually with waxy or hyaline

FIG. 36.

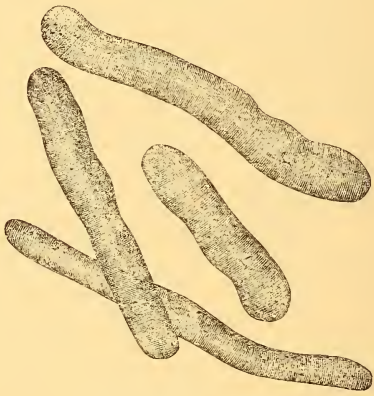


Hyaline and granular casts, illustrating the formation of the former at *a*.

casts. In consequence of the mode of formation above referred to, hyaline and waxy casts vary considerably in diameter, some being as narrow as .025 millimeter ($\frac{1}{1000}$ of an inch) and even narrower, while others are as much as .05 millimeter ($\frac{1}{500}$ of an inch) wide. There is no doubt that some of these are formed in the straight or collecting tubes near their openings on the papillæ. To these a limited number of epithelial cells is sometimes attached.

In addition to the epithelial casts above described, there are found in urine under the same circumstances moulds of the uriniferous tubules made up of simple *aggregations* of the *epithelial cells themselves*—simple exfoliations of the cellular contents of the tubule, which, having increased by proliferation, form a compact cellular mass. In addition to these are sometimes found epithelial casts in which the cells are seated on the outside or around the fibrinous mould.

FIG. 37.

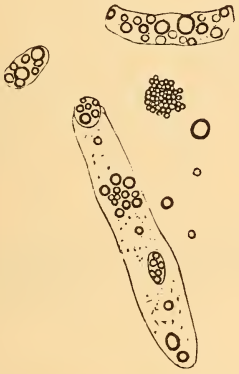


Waxy casts. × 150.

Mucus-casts.—Casts are occasionally found which are apparently pure *mucus-moulds* of the uriniferous tubules (Fig. 39). Unless covered by accidental elements, as granular urates or phosphates of lime, they are smooth, hyaline or gently fibrillated moulds, especially characterized by their great length, which is often enormous, in the course of which they divide and subdivide, diminishing in diameter as the division proceeds, showing positively that they come

from the kidney. Yet there is no albumin, or merely as much as could be accounted for by the presence of pus which sometimes attends them. For they are particularly apt to occur where there is irritation of the bladder, which is apparently extended through the ureters to the kidney. Under these circumstances they are frequently met. Dr. Beale says (*Kidney Diseases*, etc., p. 342) they are not infrequently passed in cases where the urine has a very high

FIG. 38.



Oil-casts and fatty epithelium.

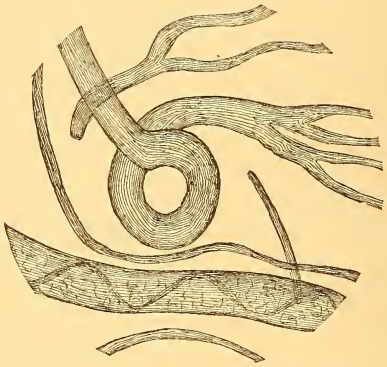
specific gravity, 1030 or higher, containing an excess of urea and urates.

These casts are not identical with the bands of mucin already alluded to (p. 203), which are found in urine of highly acid reaction. The mucin bands are probably precipitated by the acids, are often beset with granular urates, and might on this account be mistaken for casts. At the same time the mucus-cast is probably nothing but pure mucus or mucin.

Casts of the Seminal Tubules are sometimes found in the urine, but their origin may be inferred from the presence of spermatozoids in them.

To Prepare Urine for Examination for Casts.— The greatest caution should be exercised in examining urine for casts. They are often so sparsely present as to furnish no

FIG. 39.



Mucus-casts. (After Whittaker.)

deposit appreciable to the naked eye, and yet may be found by careful microscopical examination. While it is not impossible for non-albuminous urine to contain casts, yet I have met them in a few instances only, where, albumin and casts having been present, during their gradual disappearance the signs of the presence of albumin disappeared before the last casts had been washed out. On the other hand the presence of albumin means casts in the majority of instances, and many times I am certain they are declared

absent simply because they are not carefully sought. I have, however, had cases under my observation in which the urine contained large amounts of albumin, and yet by the most searching examination no casts could be found. Not a single slide, however, should satisfy the examiner, but two or three should be carefully studied throughout their entire field. Nor is a plain slide sufficient. Urine should be examined in *shallow cells*, and as those of thin glass are generally too deep, the most suitable are made with gum-damar or other suitable cement, by means of a turn-table and brush, since in this way they may be obtained sufficiently shallow to allow them to be penetrated by an ordinary one-fifth or one-fourth objective. After being made they should be put away for a month or more to thoroughly dry and harden, else they are washed off with the first cleaning of the slide.

Most casts from their lightness subside slowly, and the more so because the urine is albuminous. As soon as received, therefore, the bottle of urine should be shaken up, poured into a conical glass, and carefully covered.* Although casts generally fall to the bottom in a short time, I have known twelve hours to elapse before one could be discovered, and therefore, whenever it is possible, urine should be allowed to stand for this time in a conical glass, and then examined. If the urine has already been standing some time, the supernatant fluid may be removed, and only the lower strata containing the sediment turned into the conical glass, and allowed further to subside. A pipette,

* It is desirable that the upper surface of the conical glass be ground that it may receive one of the ground glass covers referred to on p. 17. The urine is thus thoroughly protected from the action of the air, which favors decomposition and renders the examination unsatisfactory.

made of a plain glass tube drawn nearly to a point, should then be carried to the bottom of the glass with the index finger firmly pressed upon the distal end. When it has reached the bottom, the finger should be raised and immediately returned. In this manner only the lowest drops are obtained, which are most likely to contain the casts. A drop of this fluid is allowed to fall into one of the shallow cells, covered with a thin glass cover, and carefully examined with a one-fourth or one-fifth object-glass and the No. 1 eye-piece. If these precautions are taken, and two or three slides examined, casts will either be found, or they are absent. Only the beginner need be cautioned against linen and cotton fibre, hair, or portions of deal-wood. More likely are the mucin flakes and cast-like granular aggregations of inorganic and organic matter to mislead.

V. Spermatozoids

Frequently occur in the sediment of urine of healthy men. When abundant they form a slight flocculent cloud in the urine, but there is generally nothing in the appearance of urine whence their presence may be suspected. They require a power of 400 diameters (one-fifth object-glass with the No. 2 eye-piece) to show them well, when they may be recognized by the oval head or body and the delicate tail-like prolongation emanating from it. They do not exhibit the vibratile movement after entering the urine.

Their **recognition** is most important in connection with medico-legal cases—cases of suspected rape. Their presence in vaginal mucus soon after coition, and in stains upon linen, is easy of demonstration. In the former case a drop of mucus from within the vagina is placed upon a slide, a drop of water added if necessary, covered with a

thin cover, and examined with the microscope. In the latter a simple piece of the stained linen may be soaked in water or artificial serum in a watch-glass for half an hour or an hour, and the sediment examined. Beale figures

FIG. 40.

Human spermatozoids. 1. Magnified 350 diameters. 2. 800 diameters. *a*, viewed from the side; *b*, from the front.

(Fig. 74) some filaments of a vegetable nature resembling spermatozoids.

VI. FUNGI.

Most of the living organisms found in decomposing urine, formerly looked upon as of animal origin, are now acknowledged to be vegetable in their nature, and are generally fungi.

The most frequent among these are *bacteria*, *penicillium glaucum*, and the *yeast fungus*. *Sarcinæ* are occasionally met with.

1. **Bacteria.**—In the refined study which has of late years

been given to the subject of fungi, a classification has been made of the minute objects which were formerly called monads and vibriones. They are a subdivision of Nägeli's *schyzomycetes* or cleft fungi. They include, of Cohn's classification, *a*, the *sphero-bacteria* or *micrococci*, consisting of little trembling points uniform in size and proliferating rapidly in all putrid fluids including decomposing urine; *b*, *microbacteria*, the *staff-shaped* or rod-bacteria, which appear as minute lines approaching in length with moderate powers the diameter of a red blood-disk, but mere lines in breadth, sometimes at rest and sometimes vibrating across the field; *c*, *desmobacteria*, or filamentous bacteria, including 1st, a straight form, the *bacillus*, and 2d, a curved form, *vibrio*. Bacillus increases by transverse division, and often forms a long string called *leptothrix*, which may extend entirely across the field of view. It is not constricted at the joints like the moniliform threads sometimes formed by the globular bacteria. The first two occur either *isolated* or in the so-called *zooglea form*, consisting of jelly-like masses apparently held together by a gelatinous substance. The last often form swarms but never zooglea masses.

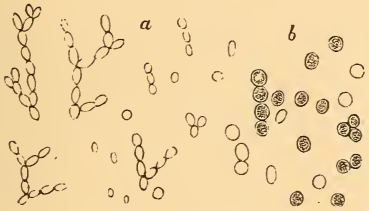
The effect of the presence of bacteria in urine is to give it a cloudiness which can be only partially removed by filtration, the bacteria being so small that they pass through the pores of the filter. They may be entirely removed by the aid of the magnesian fluid (p. 16), gentle heat, and filtration.

2. The **yeast or sugar fungus** (*saccharomyces urinæ*) consists, in the sporule-stage, of transparent oval cells, in their longer diameter about the size of a blood-disk, and of larger spherical cells, granular and nucleated. They are

found in saccharine urine, and are probably identical with the ordinary yeast fungus (*saccharomyces cerevisiæ*). The former small oval cells are often arranged in rows of two, three, or more, as seen in the figure (Fig. 41). According to Hassall, this is a fungus peculiar to saccharine urine, but the small oval cells of the sporule-stage at least cannot be distinguished from the similar stage of,

3. **Penicillium glaucum**, which occurs in acid urine with or without albumin or sugar. The sporule-stage furnishes cells very similar to those of the yeast fungus, but later, penicillium, by the union of its cells, forms thalli or

FIG. 41.



Yeast fungus. (After Harley.)

branches, which are characteristic. So, too, in the stage of aerial fructification, the penicillium multiplies by simple linear division of cells.

4. The **sarcina urinæ** is a fungus rarely met with. I have found it twice in fifteen years, once in acid urine, a second time in urine of unknown reaction. Composed of cubes, it is capable of further separation into smaller cubes. It is similar to, but smaller than, the *sarcina ventriculi* of Goodsir.

The germs of these fungi doubtless enter the urine, in the vast majority of instances, after it has passed from the

bladder, one or the other form being developed according as its germs preponderate, or according to the properties the urine may possess. Decomposition seems essential to the presence of the bacteria, but not to the other forms.

VII. The Elements of Morbid Growths.

These are seldom met in the urine. Possibly *cells* may be found, and perhaps *fragments* of the growth may be broken off, and passed with the urine. The former may be suspected to be of morbid origin by their large size, their multinuclear character, the large size of the nuclei, and diversity of the cell-forms. Spindle-cells, it must be remembered, may be derived from the ureter, urethra, and even the bladder, and must not, therefore, be considered abnormal. Indeed, every shape of cell may arise from the cells of the bladder during inflammation or irritation.

Fragments of cancerous growths which get into the urine are generally from the villous kind, and may show the capillary vessels which make up the villus, with or without the epithelial covering. Fragments suitable for examination are sometimes withdrawn with the catheter.

VIII. Entozoa.

Entozoa are seldom found in the urine in this climate. *Echinococcus* cysts, as well as their hooklets, have been passed in two or three instances recorded. The eggs and ciliated embryos of *Bilharzia hæmatobia* have been found by Dr. John Harley in three patients with the endemic hæmaturia of the Cape of Good Hope, and I had the privilege, through the kindness of Dr. S. W. Gross, of examining one of the slides containing ova, sent to this country. The parasite itself is found in the vesical, mesenteric, and portal veins, causing hæmorrhages into the intes-

tines, bladder, ureters, and pelves of the kidney. The ova and parasite are figured by Beale, op. citat., p. 402.

The *filaria sanguinis hominis*, the parasite which has recently been shown to have such an intimate association with chyluria, is sometimes found in urine.

Distoma hæmatobium has been found in the bladder, ureters, and pelves of the kidney, especially in Egypt.

The Preservation of Organized Urinary Sediments for Subsequent Examination.

Crystalline urinary sediments are so easily obtained that there is no advantage in attempting their preservation, which is always difficult. Organized sediment, on the other hand, may be preserved tolerably unaltered in several fluids. One of the simplest and best of these is a mixture of glycerin and distilled water in such proportion as to secure the average specific gravity of urine, about 1020, to which is added carbolic acid in the proportion of 1 part to 100. A weak solution of salicylic acid is also efficient, and Dr. W. W. Keen recommends a solution of chloral, ten grains to the ounce. Dr. Beale's naphtha and creasote solution is efficient, but much more troublesome to prepare.

Dr. E. S. Wood, of Boston, recommends very highly a filtered solution of acetate of potassium, specific gravity between 1050 and 1060, to which carbolic acid has been added in the proportion of 4 to 5 c.c. of the deliquesced crystal to one liter of the acetate solution. Instead of carbolic acid, salicylic acid may be added to saturation. When urine is to be transported in hot weather, or kept several days for any reason, a pinch of salicylic acid added to a four-ounce vial is generally sufficient to prevent decom-

position, and in no way impairs the reactions or alters the sediments.

To use any of these fluids, allow the sediment to subside in a conical glass, decant the supernatant fluid, replace the latter with the preservative, stir up the sediment, allow again to subside, again decant, and replace with a fresh portion of the preservative. Repeat this until the urine is thoroughly replaced by the preservative. Then place the sediment and preservative in a well-stopped ordinary vial, in which they may be kept for years without marked change; or the sediment thus permeated by the preservative may be mounted in shallow cells over which a thin glass cover is cemented.

DIFFERENTIAL DIAGNOSIS OF RENAL DISEASES.

While it is quite impossible to determine with absolute certainty, by a mere examination of the urine, all of the different affections to which the kidneys are liable, there is, nevertheless, an association, more or less close, of signs with well-determined conditions. With such association, it is important that we should be familiar, while we should as well recognize the fact that they are subject to variations and exceptions. If these facts are remembered, it is not likely that any one can be led far astray by observing the following:

I. *Acute Parenchymatous Nephritis* (*Acute Diffuse Nephritis; Scarlatinal Nephritis; Acute Tubal Nephritis*).—The urine is scanty, dark, "smoke-hued," so long as it remains acid, but becomes red if alkalized. It is highly albuminous. Its specific gravity is not constant, but apt to be high—1025

or above—not from an increase in urea, but from the presence of blood. It contains a variable but generally large amount of reddish-brown, pulverulent sediment, which, on microscopic examination, is found made up of large epithelial casts, blood-casts, hyaline casts, dark-red granular casts; also, numerous red blood-disks, and free cells from the uriniferous tubules, more or less round and nucleated, twice as wide as the blood-disks, cloudy, and more granular than in health, the granules often obscuring the nucleus. Crystals of uric acid are often present. The chlorides are first diminished, also the earthy phosphates. Hæmatin, indican, and uric acid are increased.

The patient is dropsical, much swollen about the face, and, if a child, has had scarlet fever, or, if an adult, has been exposed to rain, or has otherwise become wet while over-heated.

The disease is acute nephritis, acute diffuse nephritis, scarlatinal nephritis, or acute Bright's disease, and the chances for recovery are many.

II. *Chronic Parenchymatous Nephritis* (*Tubal Nephritis; Chronic Diffuse Nephritis; Large White Kidney*).—The urine is pale, and of low specific gravity, 1010–1015; its quantity, though variable, generally diminished. Albumin is diminished as compared with (I), but it is still abundant—one-quarter to one-half the bulk. The urine often deposits an appreciable white sediment, which by microscopic examination is found made up of black, highly granular casts, hyaline casts, and casts containing fragments of epithelium; also compound granule-cells (Fig. 33). Probably also there are casts containing a moderate quantity of oil, and perhaps also partially fatty cells. Waxy casts are also sometimes found in this form of disease. The urea is diminished, the chlorides normal, pigment diminished.

There is also often, but not always, œdema, more or less general, which may, however, subside, but the patient has a pale, almost characteristic waxy look; and towards the unfavorable termination there is much dropsy. The symptoms have existed more than six weeks.

The disease is probably the large white kidney, a chronic continuation of (I), known also as chronic tubal nephritis, and chronic diffuse nephritis, and recovery, though possible, is much less likely to occur than with the acute form.

At other times the sediment contains a large number of oil-casts filled with free oil, and oil contained in epithelial cells. There are numerous free fatty cells and free oil-globules.

III. *Secondary Contraction of the Kidney after Chronic Nephritis.*—The disease has existed for more than a year, the urine is increased as compared with (II), and may be even increased as compared with health, and the specific gravity varies accordingly. The albumin is diminished, but may be considerable, or it may be very small in amount. The urine deposits a more scanty sediment, made up of broad casts, some dark granular and others waxy, together with a few narrow pale casts. Compound granule cells occur, but are less numerous, and there may be some fatty epithelial cells, but the amount of oil, though distinctive, is not very large. The urea is much diminished. There is often some dropsy, less frequently than in (I) and (II), but more than in (IV), and it may be entirely absent.

Here the *large white kidney has probably commenced to contract*, and the resulting organ is also called the fatty and contracting kidney, to distinguish it from the next form, the chronically contracted kidney. One must be cautious about drawing too sharp a line between these (II) and (III).

The prognosis as to recovery is unfavorable, but the disease may last many years without inconveniencing the patient.

IV. *Interstitial Nephritis* (*Chronically Contracted Kidney; Granular Kidney; Red Granular Kidney; Cirrhotic Kidney.*)—The urine is increased in amount, correspondingly pale, but, while micturition may be a little more frequent, it may not attract attention. The patient may have to rise once in the night. The specific gravity is generally diminished (1010–15), while the quantity of albumin is trifling, never exceeds one-quarter, and often is shown by a mere line of opacity in Heller's test. The urine deposits often no visible sediment, and at all times a trifling one. In this are found delicate hyaline and finely granular casts, often of small diameter. Some of these contain one or two glistening oil-drops, but very minute. Here are found the casts, which are at times almost invisible. The urea is generally slightly diminished.

There is no dropsy. Hypertrophy of the left ventricle is constant, and there may be nausea and vomiting, especially in the morning, but there are often no obvious symptoms whatever connected with the disease. If any, the patient may complain of a weak, tired feeling, and this symptom should suggest an examination of the urine always. The disease may exist for years without the knowledge of the patient, who may or may not be subject to gout. Nausea is a common symptom in the advanced stages, and drowsiness a serious one. (The urine of gouty patients should be frequently examined.)

The disease is interstitial nephritis, and its product the chronically contracted kidney. If exposure to cold and fatigue be avoided, the patient's life may be scarcely shortened, and yet he is constantly liable to attacks of

uræmia, which may suddenly terminate his life, and in advanced stages of the disease the cardiac symptoms often produce great discomfort.

V. *Lardaceous or Amyloid Degeneration of the Kidney.*—The urine is increased in quantity, clear, of corresponding specific gravity (1007–1015), of a pale golden color, the color of a dilute urine only, contains at first little, but later considerable albumin, about one-fourth to one-half; urea is diminished. There is very little or no sediment visible. Casts are often wanting, and when present include the broad dark granular as well as the hyaline and waxy casts; occasionally fatty casts are found; the waxy are solid-looking, and *sometimes* give the characteristic red reaction of the amyloid substance when treated with a *watery* solution of iodine and iodide of potassium. Here hyaline and waxy casts of large diameter are found, and sometimes within these smaller casts.

While the highly refracting waxy casts are not confined to albuminoid kidney, they always indicate chronic and deep-seated processes.

At first there is no dropsy, but later it is sometimes persistent. Generally, however, except towards the termination of the case, it is amenable to treatment by rest and diuretics. The patient has an enlarged liver or spleen, sometimes obstinate diarrhœa; he has had syphilis, or extensive disease of the bones, or has phthisis.

The disease is lardaceous degeneration of the kidney, and is incurable, though the patient may live many years.

VI. *Acute Active Hyperæmia* (*Parenchymatous Degeneration of the Kidney; "Cloudy Swelling"*).—Most frequently the sole symptom is albuminuria, the most careful examination failing to discover casts; when casts are present, they

are of the hyaline variety. There is, as a rule, no dropsy. The quantity of albumin ($\frac{1}{10}$ to $\frac{1}{4}$ bulk) is generally less than in tubal or parenchymatous *inflammation* or lardaceous degeneration. Such may sometimes be the albuminuria of pregnancy, or such grave diseases as diphtheria and acute febrile disorders.

After death, the renal epithelia are often more or less enlarged, their contents cloudy. This condition differs from parenchymatous *nephritis* in the smaller extent and diminished intensity of the morbid process. It is probably due to the pernicious influence of some poison on the minute structure of the kidney, which may extend to all the tissues. Recovery is frequent.

The disease was called by Niemeyer *parenchymatous degeneration.*

VII. *Cyanotic Induration.*—Cyanotic induration is a peculiar indurated form of kidney due to a simple hyperplasia of its interstitial tissue, the result of long-continued passive congestion. It is most frequently found accompanying valvular disease of the heart, and is characterized by a bluish color.

In addition to the other symptoms of heart disease there is dropsy and often serous effusion in the great cavities. The urine is scanty, of high specific gravity, often 1030 and above, there is usually a moderate amount of albumin, and a few small hyaline or faintly granular casts; but casts are often absent.

The prognosis is not good, but under favorable circumstances the patient may improve and become more comfortable.

The above is given as a general guide, and I would again refer to the fact that there are deviations from the condi-

tions laid down. There are still many points quite disputed in the pathology of the kidney. Thus, the older German pathologists contended that there is a constant relation of succession between the acute parenchymatous nephritis, the chronic parenchymatous nephritis (large white kidney), and the contracting stage of the latter, making no distinction between the cirrhotic kidney and the fatty and contracting kidney. And it is held by some to-day that all inflammations of the kidney are *diffuse;* that is, there is no inflammation in which the epithelial tissue or the connective tissue alone is primarily involved, but that both always share the process from the beginning.

One more fact must be mentioned in this connection, and this is that although the presence of fatty casts and fatty epithelium is an unfavorable symptom, yet it does not follow that such cases are necessarily fatal. I have, on more than one occasion, found oil-casts in the urine of patients, and yet have also found them to disappear altogether. The circumstances under which this has most frequently occurred have been, 1st, where there have been heart disease and kidney disease combined, and there has been some exacerbation of one or both, when the albumin has increased, and oil-casts have made their appearance, which later totally disappeared; 2d, where pregnancy has supervened on existing Bright's disease, and oil-casts have been present, which again disappeared after a successful labor.

PART III.

URINARY CALCULI.

THE qualitative analysis of gravel or calculus is much simpler than is generally supposed. There are but three varieties of calculus at all common, and therefore likely to demand analysis. These are (1) *uric acid and its compounds*, (2) *oxalate of lime*, and (3) *the mixed phosphates*. Calculi of *xanthin* and *cystin* occur, though very rarely.

1. **Uric acid calculi** are the most common. They are either red or some shade of red, and usually smooth, but may be tuberculated. They leave a mere trace of residue after ignition.

2. **Oxalate of lime calculi** are frequently met with. They are generally of a dark-brown or dark-gray color, and from their frequently tuberculated surface have been called mulberry calculi. They may, however, be smooth when small—hemp-seed calculi. Considerable residue remains after ignition. The calculus is soluble in mineral acids without effervescence.

3. **Calculi of the mixed phosphates,** or **fusible calculi,** are composed of the phosphate of lime and of the triple phosphate of ammonium and magnesium. Phosphates make up the external layers of many calculi of various composition, and may form entire calculi, but seldom constitute the nucleus alone, of a calculus. Mixed phosphatic calculi are white, exceedingly brittle, fuse in the blowpipe flame and are soluble in acids, but insoluble in alkalies.

Other rarer forms of calculi are made up of **carbonate of lime**, of **xanthin**, **cystin**, and of **urostealith.**

Few calculi of large size are of the same composition throughout, and when of any considerable size, these constituents are usually arranged in concentric layers about a nucleus. Oxalate of lime is the most frequent nucleus; uric acid may also serve as a nucleus, but phosphates, almost never. Small masses of organic matter, as blood-clots, frequently form nuclei, and may often be recognized by the odor of ammonia on ignition. Foreign bodies, as pieces of pencil or even glass, introduced into the bladder from without, may become nuclei.

To Determine the Composition of Calculi Qualitatively.

Previous to chemical treatment a calculus should be *powdered*, and in view of the fact that different layers are often of different composition, wherever the stone is of any size, it should be sawed or coarsely broken and a portion of each layer should be examined separately.

Expose a portion of the powdered calculus on a piece of platinum foil or a platinum spoon to a dull red heat for a considerable time. Note whether there is a residue.

A calculus which burns with very little or no fixed residue is composed either of uric acid or ammonium urate, of cystin, of xanthin, or of urostealith. If it does not burn completely it may contain uric acid and uric acid salts, phosphate of lime and phosphate of magnesium, the ammonio-magnesian phosphate, or oxalate of lime.

A. There is a fixed residue. To a portion of the original powder apply the murexid test (p. 160).

I. A purple color results: *uric acid* is present.

Observe whether a portion of the calculus melts on being heated.

a. It melts, and communicates—

1. A strong yellow color to the flame of a spirit-lamp, or Bunsen burner: *sodium urate.*
2. A violet color to the flame: *potassium urate.*

b. It does not melt. Dissolve the residue after ignition in a little dilute HCl, add ammonia until alkaline, and then ammonium carbonate solution.

1. A white precipitate falls: *calcium urate.*
2. No precipitate. Add some hydric sodic phosphate solution; a white crystalline precipitate falls: *magnesium urate.*

II. No purple color results.

Observe whether a portion of the calculus melts on being exposed to the *blow-pipe* flame.

a. It melts (*fusible calculus*). Treat the residue with acetic acid; it dissolves. Add to the solution ammonia in excess; a white crystalline precipitate falls: *ammonio-magnesium phosphate.* In case the melted residue is insoluble in acetic acid, treat with HCl; it dissolves. Add to the solution ammonia; a white precipitate indicates *calcium phosphate.* Ammonio-magnesian phosphate and basic calcium phosphate ordinarily occur mixed in the same concretion.

b. It does not melt. Moisten the residue with water, and test its reaction with litmus-paper; it is not alkaline. Treat with HCl; it dissolves without effervescence. Add to the solution ammonia in excess; white precipitate; *calcium phosphate.* Calculi of pure calcium phosphate are rare, but may occur.

Treat the powdered calculus with acetic acid; it does not dissolve. Treat the residue, after heating, with acetic acid; it dissolves with effervescence: *calcium oxalate*.* The powdered original calculus dissolves with effervescence when treated with acetic acid; *calcium carbonate*.

B. *There is no fixed residue.* Apply the murexid test (p. 160).

I. A purple color is developed.

a. Mix a portion of the powdered calculus with a little lime, and moisten with a little water; ammonia is evolved, and a red litmus-paper suspended over the mass is turned blue: *ammonium urate*.

b. No ammonia: *uric acid*.

II. No purple color.

a. But the nitric acid solution turns yellow as it is evaporated, and leaves a residue insoluble in potassium carbonate: *xanthin*.

b. The nitric acid solution turns dark brown, and leaves a residue soluble in ammonia: *cystin*.

c. The calculus, soft when fresh, dark-brown and brittle when dry, becomes softer again when warmed. Soluble in ether, the amorphous mass, after evaporation of the ether, becomes violet on being heated. It dissolves in nitric acid, with slight evolution of gas and without change of color: *urostealith*.

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* The calcium oxalate is converted by the heating into calcium carbonate, which dissolves with effervescence. If the heat be much higher than a dull red, the carbonate of lime is converted into quicklime, which does not effervesce on adding an acid.

A coarser mode of analysis, but still sufficiently accurate for practical purposes in most instances, is the following:

Powder a portion of the calculus and ignite upon a piece of platinum foil.

A. There is a fixed residue. To a portion of the powdered original calculus apply the murexid test (p. 160):

I. A purple color results. The stone is composed of *uric acid* or *uric acid and its compounds*.

II. No purple color results.

Observe whether a portion of the original calculus melts under the flame of the blowpipe.

a. It melts (fusible calculus). The stone is the *ammonio-magnesian phosphate*, containing, also, probably, some phosphate of lime.

b. It does not melt. (1) Treat some of the original calculus in powder with acetic acid. It does not dissolve. Treat the residue, after heating, with acetic acid. It dissolves with effervescence. The stone is *oxalate of lime*.

(2) The powder of the original calculus dissolves with effervescence. The stone is *calcium carbonate*.

B. There is no fixed residue. Apply the murexid test (p. 160).

I. A purple color results. The stone is *uric acid* or *uric acid and its compounds*.

II. No purple color. (See II, p. 238.)

APPENDIX.

MODE OF RECORDING AN EXAMINATION.

To systematize and facilitate the work of urine examinations, forms of record have been devised by those working in the subject. For ordinary use in hospital and private practice a modification of the form suggested by Heller recommends itself for its economy and convenience.

The form may be printed, but as recommended by Heller, an ordinary half-sheet of letter-paper is folded in four, and marked in the manner indicated below:

PHYSICAL PROPERTIES.

Quantity taken in twenty-four hours.
Color and reaction.
Sp. gr., quantity and character of sediment.

NORMAL CONSTITUENTS.

Uph. (Urophain).	Cl. (Chlorides).
Ux. (Uroxanthin).	Eph. (Earthy phosphates).
$\overset{+}{\text{U}}$. (Urea).	Alkaline phosphates.
$\overline{\text{U}}$. (Uric acid).	Sulphates.

ABNORMAL CONSTITUENTS IN SOLUTION.

SEDIMENT.

CONCLUSION.

Abbreviations for the important constituents are used as shown, the sign "+" for *increased*, the sign "—" for *diminished*, and the letter "n." for *normal*. For *great* increase or *great* diminution, "gr. +" and "gr. —" may be used, and for *slight* increase or *slight* diminution, "sl. +" or "sl. —."

I have added another space for the opinion or diagnosis.

Let us suppose an examination to have been made, with the following results. The word "indican," "*ind.*," is preferred for "uroxanthin," and substituted.

PHYSICAL PROPERTIES.

Quantity in twenty-four hours, 500 c.c.
Color, very pale yellow. Reaction, acid.
Sp. gr., 1005. Sediment, moderate, flocculent.

NORMAL CONSTITUENTS.

Uph. gr. —		Cl.	n.
Ind. sl. +		Eph.	—
$\frac{+}{U}$ / $\frac{}{U}$	gr.—	Aph. / Sph.	—

ABNORMAL CONSTITUENTS IN SOLUTION.

Albumin, 2 per cent.

SEDIMENT.

Numerous oil-casts, free fatty cells and free oil-globules.

Diagnosis—Chronic Parenchymatous nephritis.
Fatty kidney.

TABLES

For Reducing the Metric or French System into the English, and vice versa, as far as required in Urinalysis.

Grams to Grains.			Grains to Milligrams.		
1	=	15.43 (+ .0022)	1	=	64.8 (— .000425)
2	=	30.86	2	=	120.6
3	=	46.29	3	=	194.4
4	=	61.72	4	=	259.2
5	=	77.15	5	=	324.0
6	=	92.58	6	=	388.8
7	=	108.01	7	=	453.6
8	=	123.44	8	=	518.4
9	=	138.87	9	=	583.2

Cubic Centimeters to Minims.			Minims to Cubic Centimeters.		
1	=	16.2 (+ .0293)	1	=	.0616
2	=	32.4	2	=	.1232
3	=	48.6	3	=	.1848
4	=	64.8	4	=	.2464
5	=	81.0	5	=	.3080
6	=	97.2	6	=	.3696
7	=	113.4	7	=	.4312
8	=	129.6	8	=	.4928
9	=	145.8	9	=	.5544

Cubic Centimeters to Fluidrachms.			Fluidrachms to Cubic Centimeters.		
1	=	.27 (+ .0005)	1	=	3.7
2	=	.54	2	=	7.4
3	=	.81	3	=	11.1
4	=	1.08	4	=	14.8
5	=	1.35	5	=	18.5
6	=	1.62	6	=	22.2
7	=	1.89	7	=	25.9
8	=	2.16	8	=	29.6
9	=	2.43	9	=	33.3

Liters to Fluidounces.

1	=	33.8 (+ .011)
2	=	67.6
3	=	101.4
4	=	135.2
5	=	169.0
6	=	202.8
7	=	236.6
8	=	270.4
9	=	304.2

Fluidounces to Cubic Centimeters.

1	=	30 (— .4238)
2	=	60
3	=	90
4	=	120
5	=	150
6	=	180
7	=	210
8	=	240
9	=	270

Liters to Pints.

1	=	2.1 (+ .013188)
2	=	4.2
3	=	6.3
4	=	8.4
5	=	10.5
6	=	12.6
7	=	14.7
8	=	16.8
9	=	18.9

Pints to Liters.

1	=	.473 (+ .00022)
2	=	.946
3	=	1.419
4	=	1.892
5	=	2.365
6	=	2.838
7	=	3.311
8	=	3.784
9	=	4.257

Inches to Millimeters.

1	=	25.4 (+ .00005)
2	=	50.8
3	=	76.2
4	=	101.6
5	=	127.0
6	=	152.4
7	=	177.8
8	=	193.2
9	=	228.6

Millimeters to Inches.

1	=	.03937
2	=	.07874
3	=	.11811
4	=	.15748
5	=	.19685
6	=	.23622
7	=	.27559
8	=	.31496
9	=	.35433

Meters to Feet.			Feet to Meters.		
1	=	3.28	1	=	.3048 (+ .0000005)
2	=	6.56	2	=	.6096
3	=	9.84	3	=	.9144
4	=	13.12	4	=	1.2192
5	=	16.40	5	=	1.5240
6	=	19.68	6	=	1.8288
7	=	22.96	7	=	2.1336
8	=	26.24	8	=	2.4348
9	=	29.52	9	=	2.7432

To Convert Degrees of Fahrenheit's Thermometer to Centigrade, and vice versa.

Centigrade to Fahrenheit.			Fahrenheit to Centigrade.		
1	=	1.8	1	=	.555 (+ .000555)
2	=	3.6	2	=	1.110
3	=	5.4	3	=	1.665
4	=	7.2	4	=	2.220
5	=	9.0	5	=	2.775
6	=	10.8	6	=	3.330
7	=	12.6	7	=	3.885
8	=	14.4	8	=	4.440
9	=	16.2	9	=	4.995

To use this table, convert the given number of degrees Centigrade into degrees Fahrenheit, and add 32°.

To use this table, subtract 32° from the given number of degrees Fahrenheit, and convert the remainder into degrees Centigrade.

(*From Dr. Craig's Decimal System.*)

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